NATURE

No. 4787 SATURDAY, JULY 29, 1961 Vol. 191

CONTENTS

	Page
Conservation of Water in Great Britain	421
Achievements in Physics. By Prof. W. E. Burcham, F.R.S.	423
A New Approach to General Relativity. By Dr. G. J. Whitrow	424
New Handbooks for Lunar Studies. By D. H. P. Jones	424
Issues in Evolution. By Dr. P. G. Fothergill	425
Heterocyclic Chemistry. By Dr. G. L. Buchanan	425
Coastal Inlets. By W. J. Reid	426
Mathematical Studies in the Psychology of War. By Sir Cyril Burt	427
Science in the Himalaya. By Dr. L. G. C. E. Pugh	429
The Chester Beatty Research Institute (of the Institute of Cancer Research: Royal Cancer Hospital). By Prof. A. Haddow, F.R.S.	430
Metabolic Production of Sucrose from Fat. By Prof. H. Beevers	433
Radiocæsium and Potassium in Norwegians. By Johan Baarli, Kjell Madshus, Kurt Lidén and Richard C. McCall	436
News and Views	439
Water Resources of the United States. By Prof. W. G. V. Balchin	444
Flora Europæa. By Dr. V. H. Heywood	446
European Broadcasting Conference, Stockholm	449
The British Electrical and Allied Industries Research Association. By Prof. J. Greig	450
Four Decades of State Forestry in New Zealand	451
The Case for a New Profession embracing Science, Librarianship and Foreign Languages	452
Distribution of Brightness in Extragalactic Radio Sources. By A. T. Moffet and P. Maltby	453
Comparison of Beryllium-7 and Cæsium-137 Radioactivity in Ground-level Air. By Dr. P. F. Gustafson, M. A. Kerrigan and S. S. Brar	454
Sustained Oscillations in a Catalytic Chemical System. By R. A. Spangler and F. M. Snell	457
Minimum Energy Theorem for Flow of Dry Granules through Apertures. By R. L. Brown	458
Comparative Analysis of Inactivating Efficiency of Radiation on Different Organisms. By Dr. M. Terzi	461
Formation and Utilization of Poly-β-Hydroxybutyric Acid by Knallgas Bacteria (*Hydrogenomonas*). By Prof. H. G. Schlegel, G. Gottschalk and R. von Bartha	463
Apparent Cytoplasmic Inheritance of Tyrosinase Competence in *Streptomyces scabies*. By Prof. K. F. Gregory and Wei-Jen Shyu	465
Comparison of Pyridine Nucleotides in the Liver and in an Ascitic Hepatoma. By M. Wintzerith, N. Klein, L. Mandel and Prof. P. Mandel	467
Biochemical Aspects of Resistance to 6-Mercaptopurine in Human Epidermoid Carcinoma Cells in Culture. By R. W. Brockman, George G. Kelley, Patricia Stutts and Virginia Copeland	469
Limits of Human Vision. By Dr. R. A. Weale	471
Phosphatases in *Schistosoma mansoni*. By Dr. D. L. H. Robinson	473
Social Dominance in Inbred Mouse Strains. By Prof. G. Lindzey, H. Winston and M. Manosevitz	474
Significance of the Anti-Sporulation Effect of Copper Fungicides in the Control of Sigatoka Disease of Bananas caused by *Mycosphærella musicola* Leach. By E. Evans, H. L. S. Bevington and E. P. Whitaker	476
Age of Bed I, Olduvai Gorge, Tanganyika. By Dr. L. S. B. Leakey, Dr. J. F. Evernden and Dr. G. H. Curtis	478
Recent Scientific and Technical Books	Supp. iv

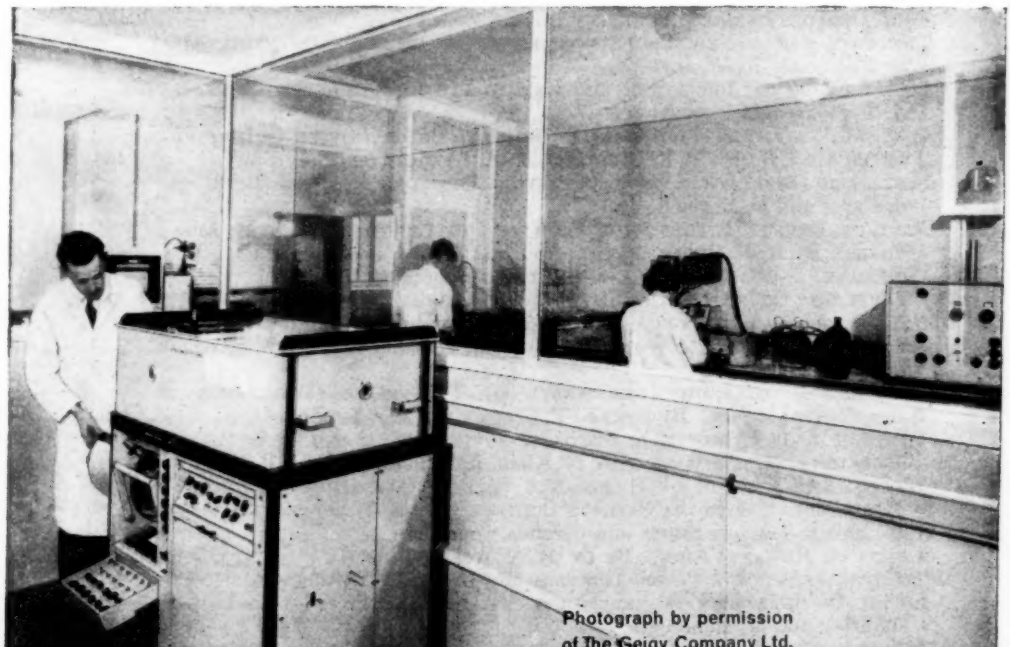
(*Continued overleaf*)

UNICAM

The highest levels of accuracy and reliability are essential to the quality control service of the pharmaceutical industry. The Unicam SP.100 Infrared Spectrophotometer provides more information in less time and with less effort—to performance levels previously associated only with research equipment built to specialist specification.

Detailed attention to operating systems and a comprehensive monitoring routine allow even the unskilled operator to achieve the outstanding performance offered by the SP.100. For information on how the SP.100 can help in your laboratory please write to: Unicam Instruments Limited, York Street, Cambridge.

SP.100 Infrared Spectrophotometer



Photograph by permission of The Geigy Company Ltd.

Other Unicam instruments appearing in this photograph are the SP.500 Ultraviolet Visible and Near Infrared Spectrophotometer and the SP.900 Flame Spectrophotometer.

UNICAM INSTRUMENTS LTD · YORK STREET · CAMBRIDGE

LETTERS TO THE EDITORS

Page

SPACE SCIENCE

Interpretation of Cosmic Noise Measurements at 3·8 Mc./s. from Satellite 1960 η1.—J. H. Chapman and A. R. Molozzi 480

ASTROPHYSICS

Apparent Recession of Nebulæ and the Nature of Radio Stars.—Dr. N. S. Japolsky 480

GEOPHYSICS

The Frequency of Meteorite Falls throughout the Ages.—Prof. Herbert Dingle; Prof. Hans Pettersson, For.Mem.R.S. 482

RADIOPHYSICS

Scintillations and the Latitude Distribution of Ionospheric Irregularities.—D. G. Singleton . . . 482

PHYSICS

Pulse-shortening in Electron Linear Accelerators.—Dr. M. C. Crowley-Milling, T. R. Jarvis, C. W. Miller and G. Saxon 483

Displacement Potentials and Bending of Rod-like Polyelectrolytes.—Prof. J. A. Christiansen, (the late) Dr. C. E. Jensen and Dr. Th. Vilstrup . . . 484

Electrical Effects observed in Polycrystalline Boron.—C. H. Carmichael and M. Dore 485

GEOLOGY

Age of the Orogeny and Granites in South-West England.—T. R. Owen; N. E. Butcher . . . 486

CHEMISTRY

Evaporated Carbon Films of Large Area for Adsorption Studies by Electron Bombardment.—A. E. Bell, J. Pritchard and Prof. K. W. Sykes 487

The Marangoni Effect and Liquid/Liquid Coalescence.—G. D. M. MacKay and S. G. Mason 488

Formation of Calcium Chloraluminate by Interaction of Portland Cement with Sea-water and Chloride Solutions.—Dr. L. Heller and Dr. M. Ben-Yair . 488

Chromium, Uranium, Tungsten and Nickel Complexes of Thioglycolic Acid.—V. M. Bhuchar . . . 489

BIOCHEMISTRY

Interaction of Hydrocortisone, the Sulphydryl Group, and the Catechol Amines.—Dr. Sidney Roston . 490

Effects of Injections of Some Intermediary Metabolites on the 'Insulin Response' of Normal Rabbits.—Dr. H. D. Brahmachari and G. Raghupathy Sarma . 491

Estimation of Protein in Cellular Material.—Prof. David Racusen and D. B. Johnstone 492

1-(Indole-3-acetyl)-β-D-Glucose, a New Compound in the Metabolism of Indole-3-acetic Acid in Plants.—Dr. M. H. Zenk 493

Effect of Amino-Acid Esters on the Growth of Fibroblast Cultures.—Dr. I. Fehér, A. Döklen and V. Selmeci 494

A Convenient Synthesis of Muramic Acid and Other 3-O-Ethers of D-Glucosamine.—Dr. Roy Gigg and Patricia M. Carroll 495

Differential Inhibition of Human Serum Cholinesterase with Fluoride: Recognition of Two New Phenotypes.—Prof. H. Harris and Mary Whittaker 496

The First Anthocyanins appearing during the Ripening of Blueberries.—Dr. Heikki Suomalainen and A. J. A. Keränen 498

PHYSIOLOGY

Effect of Administration of Lysergic Acid Diethylamide on Serotonin-Levels in the Body.—Dr. D. V. Siva Sankar, D. Barbara Sankar, Edward Phipps and Eleanor Gold 499

Effect of the Bilateral Section of the Splanchnic Nerve on Erythropoiesis.—Fumimaro Takaku, Kunitake Hirashima and Prof. Shigeo Okinaka 500

Dependence on Substrate of the Electrical Potential across the Isolated Gut.—Dr. T. W. Clarkson, A. C. Cross and S. Toole 501

Soluble Material in the Gastro-intestinal Tract of Rats under Normal Feeding Conditions.—Dr. Anne S. Cole 502

HÆMATOLOGY

Stability of Freeze-dried Anti-hæmophilic Globulin.—F. Nour-Eldin 305

New *Rh* Phenotype, *Dcc/cf*, found in a Chibcha Indian Tribe.—Dr. Miguel Layrisse, Zulay Layrisse, Esperanza García, Dr. Johannes Wilbert and Joaquín Parra R. 503

HISTOCHEMISTRY

Direct Relationship of Phosphorylase and Mitochondrial α-Glycerophosphate Dehydrogenase Activity in Skeletal Muscle.—Dr. A. G. E. Pearse 504

HISTOLOGY

Deoxyribonucleic Acid Content of Nuclei dividing amitotically.—Prof. K. W. Cleland 504

Photo-Sensitivity of Thiazine Leucobases.—D. W. Menzies 505

PATHOLOGY

Effects of *Grewia populifolia* Extracts on the Development of Chick Embryos.—S. K. Brahma and N. Kundu 506

Pathological Parthenogenesis in a Viviparous Toothcarp.—Prof. A. Stolk 507

Diagnostic Significance of Heterogeneous Lactic Dehydrogenases in Malignant Effusions.—R. Richterich, K. Zuppinger and E. Rossi 507

An Antigen in Lesions of Molluscum Contagiosum.—Dr. William L. Epstein, Irene P. Senecal and Dr. Alan M. Massing 509

Leukæmia in the Progeny of Mice inoculated with a Leukæmogenic Virus.—Dr. M. H. Salaman and J. J. Harvey 509

IMMUNOLOGY

Antibodies to Trophoblasts during the Post-Partum Period.—J. F. Hulka, K. C. Hsu and S. M. Beiser . 510

Specific Inhibition of Anti-*D* Antibody by Colominic Acid.—Prof. William C. Boyd and Egli Reeves 511

BIOLOGY

Hydrogen Ion Concentration, Plankton and Fish in Fresh-water Eutrophic Lakes of India.—Dr. S. M. Das 511

Littoral Interstitial Species from Anglesey representing Three Families new to Britain.—P. J. S. Boaden . 512

Response to Series of Subliminal Stimuli; an Ethological Method for investigating the Properties of Nervous Systems.—A. J. Pontin 512

Enhanced Plant-Growth with Two Systemic Insecticides.—M. M. H. Wallace 513

Effects of Gibberellic Acid and Allogibberic Acid on Flowering and Internode-Length in *Pisum sativum*.—I. C. Murfet and H. N. Barber 514

Susceptibility to Leaf Blight, caused by *Cochliobolus heterostrophus*, in Nigerian Varieties of Maize.—C. L. M. van Eijnatten 515

Diurnal Variation in Transaminase Activity in the Cactus Plant.—S. K. Mukerji, G. G. Sanwal and Prof. P. S. Krishnan 516

Shortening the Juvenile Phase of Apple Seedlings.—A. I. Campbell 517

Cactus Virus in the United States.—(The late) Irene M. Sammons and Prof. M. Chessin 517

Antimicrobial Substances from Ferns.—Prof. Jasper C. Maruzzella 518

Introduction of the Potato into Western and Central Europe.—V. S. Lekhnovitch 518

ENTOMOLOGY

Air-Swallowing: a Mechanism in Photic Reversal of the Beetle *Trypodendron*.—Prof. Kenneth Graham . 519

MICROBIOLOGY

Oxygen Inhibition of Respiration in *Azotobacter*.—M. J. Dilworth and C. A. Parker 520

A Micro-method for performing the Phenylalanine Test on Cultures from Multi-carbohydrate Media.—D. J. Stewart 521

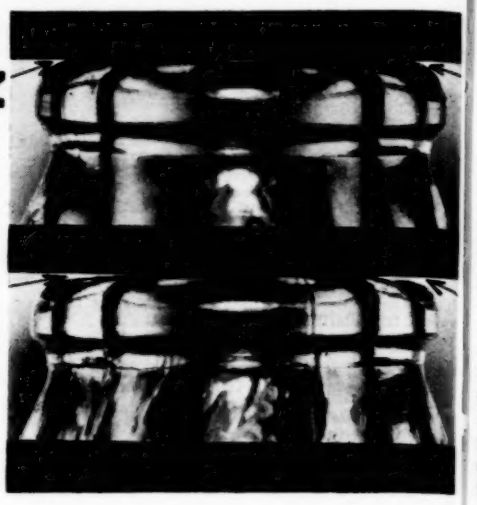
Absence of Lysogeny in *Pasteurella pestis*.—Dr. D. A. Smith 522

GENETICS

Effect of Hybridization on the Primary Polydipsic Trait of an Inbred Strain of Mice.—Dr. Emanuel Silverstein 523

Chromosome Rearrangements induced by X-Rays in Immature Germ Cells of *Drosophila*.—Seymour Abrahamson 523

FIZZ



Shortly after a new soft drink came on the market it was discovered that the gas content was escaping. Quite why, was a problem for some time. The most likely reason was that the cork was not fitting properly — but how to find out? Douglas Lawson, F.I.B.P. photographed the tops of sound and unsound bottles of the soft drink with a ½ lb. piece of metal, ground to 1/1000″ flatness tolerance, across the top. The photomacrographs on Kodak 0.250 Plates recorded gaps and bad finishing at the mouth of the bottles. You can see the difference between a good and bad bottle in the photographs — a good bottle is shown at the top with a bad one below. Another problem solved by photography and Kodak photographic materials.

Kodak 0.250 PLATES

Kodak Limited, Industrial Sales Division,
Kodak House, Kingsway, London, W.C.2.

HIGH PERFORMANCE in the MEDIUM PRICE RANGE is the VA17A ACHIEVEMENT

1. The VA17A Automatic Memory* Calculator retains any factor **during operation** for later use as a constant divisor, dividend, multiplier etc.
2. Extreme simplicity of control — the VA17A can be 'programmed' if work has to be delegated.
3. Fully automatic in **all** operations including negative multiplication and division, zero skipping and automatic squaring.
4. Direct addition in **multiplier** register enabling immediate multiplication of totals — or two totals simultaneously.
5. Direct calculation of intermediate answers without re-setting — a speedy solution to difficult problems.
6. Correct decimal positioning, full tens transmission and so much more than you expect at £285.

Badenia
CALCULATORS LIMITED
LION HOUSE, 72 RED LION ST., W.C.1. HOL 7744

Please send me details of:
- ☐ VA 17 CALCULATORS
- ☐ DIA-PLAN WALL CHART
- ☐ ROL-A-CHART WALL CHART
- ☐ NORWEGIAN "MINIATURE OFFICE" in a cabinet

Name .....
Firm .....
Address .....

CONSERVATION OF WATER IN GREAT BRITAIN

WATER has been the key to man's advancement since before the beginning of recorded history. Not only do we need it for personal use, but it is also essential to grow crops, to water stock, to move cargoes, and to dispose of wastes. Many manufacturing processes depend on this precious liquid: it is the universal solvent, the universal coolant. It powers part of industry, and when work is done it is essential to much recreational activity. Without water, life as we know it would cease. To-day, this vital raw material is assuming an ever-increasing importance as the need for it and water-related services and products grow. Inconvenience arises by both an absence or over-abundance of water. The conservation of water therefore becomes of importance whenever extremes are experienced, or when the demand for water by a growing community begins to exceed the minimum supply available. There has been for some years now mounting evidence that we are rapidly approaching a time when the latter situation will arise in Great Britain.

In 1959, as a result of a dry summer, some ten million people (nearly one quarter of the population of England and Wales) were affected by drought orders or restrictions on their water supply. Moreover, although 1959 had a remarkably dry summer the year as a whole was not exceptionally dry—there have been ten drier years in the present century, notably in 1955. These restrictions demonstrated the already known fact that Great Britain's defences against drought have been a diminishing quantity for some time[1]. On the other hand, the above-average rainfall in the autumn of 1960, coming after a wet summer, led to very serious and prolonged flooding in a number of areas. These experiences now seem to have convinced the central Government authorities that there is an increasing need to regard water as a prime natural resource basic to our existence, and to legislate for its conservation and proper use.

The past year or so has seen the welcome appearance of a number of valuable reports and publications, notably the hydrological surveys and the annual year books issued by the Surface Water Survey[2], together with the reports of the Central Advisory Water Committee[3] and the Welsh Advisory Water Committee[4]. The British National Committee for Geodesy and Geophysics has also issued a useful report on current hydrological activity in the United Kingdom[5].

Valuable as these reports are, it must be admitted that they do not match up to the remarkable study of the same problem undertaken in the United States during the past two years by the Senate Select Committee on National Water Resources[6]. The British reports, however, represent a significant step in the right direction, and accompanying them have been welcome indications that the Ministry of Housing and Local Government is now preparing the way for new legislation to meet the future water needs of the country. In June 1960 the Minister stated: "We are approaching the point at which we may very possibly have to take a new look at water conservation. . . . It may be that the time is not far distant when we have to look at all the resources of a river basin as a whole in the widest possible way, to make sure that all the various and sometimes conflicting interests are properly served; that the river is kept clean and the interests of fishing, navigation and so on are safeguarded, while, at the same time, the maximum amount of water is made available to all kinds of users as economically as possible".

Since this announcement, one of the sub-committees of the Central Advisory Water Committee has made an investigation into the complex problems involved in achieving this end, and the Committee is now engaged on the preparation of a report. In view of the public concern and of the wide support that can be expected for a bold approach, the Minister has decided to open confidential discussions with representatives of affected interests, both private and public, in advance of the report. An indication has therefore recently been given by the Ministry of the proposals which it has in mind as a basis for legislation dealing with water conservation.

There has been support in some quarters for a Central Water Authority mainly concerned with policy and planning, but also possessing powers of direction. It has been suggested that such an authority should have a regional organization with executive powers, and ideas on its constitution have varied from a body such as the Central Advisory Water Committee with extended functions, to a newly appointed permanent body of persons with specialist experience and knowledge in water problems. This proposition has been considered, but has been rejected by the Minister on grounds of constitutional and administrative difficulties.

It is proposed instead to set up conservation authorities and to re-define the powers and responsibilities of the Minister. Section 1 of the Water Act, 1945, requires the Minister "to promote the conservation and proper use of water resources and the provision of water supplies in England and Wales and to secure the effective execution by water undertakers under his control and direction, of a national policy relating to water". The present statutory provisions, however, are ill-suited for the execution of this policy: thus the only specific provision to enable conservation to be carried out is to be found in section 14 of the Act, which empowers the Minister to control new abstractions of underground water in those parts of the country designated as conservation areas. So far as surface water is concerned, there is no provision whereby both the development and allocation of supplies can be controlled in the light of an overall plan which has regard to all the various interests involved. It has been clear to many and for some time now that if we are to make the best use of our water resources and due regard is to be

paid to future needs, then a completely new comprehensive approach is urgently needed.

The Minister has accepted that any new approach should be based on certain propositions which are beyond argument. These are briefly: (*a*) that the approach to the problem should be by way of the natural hydrological unit of the river basin; (*b*) that surface and underground water are interdependent and any measures for development and control must be related to both; (*c*) that the machinery of conservation must be capable of taking into account all the conflicting interests; (*d*) that regulatory powers alone are insufficient and that a positive policy involving new works will in certain cases be necessary; (*e*) that a need will exist for some central co-ordinating and appellate authority.

It is therefore proposed to establish water conservation authorities (their exact title has yet to be decided) founded on river basins or groups of river basins. The question immediately arises whether the new organizations should be set up in parallel with the existing river boards. In the Minister's view, conservation functions and those at present carried out by river boards are so interdependent that they should be the responsibility of a single authority in each area.

It is thought that the new conservation authorities should be responsible for: (*a*) keeping a complete up-to-date hydrological survey of the area; (*b*) the preparation of an overall plan for the development of the water resources; (*c*) the control and development of the resources; (*d*) the allocation of water between users by means of a licensing system, subject to a right of appeal to the central authority; (*e*) the recharge of underground aquifers, the discharge of compensation water and the transfer of water between conservation areas; (*f*) land drainage, flood prevention and the control of pollution; (*g*) the administration of salmon and freshwater fisheries legislation, and the reconciliation of navigational needs with the above interests.

The new approach is therefore comprehensive, and all water-using interests in a particular river basin would be brought under the one umbrella of the conservation authority. This is, in fact, the logical pattern which has been and is being followed in the development of many new overseas territories. The existing system within England and Wales has grown up piecemeal over the centuries, and we now have a great multiplicity of bodies concerned with all the above aspects, and the boundaries of which rarely reflect the basic hydrological and geographical facts. The transformation will clearly not be achieved without some opposition and will take some time. It is important, however, that progress on these lines should be made in order to ensure adequate supplies of water for future generations.

The financial aspects of the proposals are likely to be complex and will need careful thought. It is proposed to hold discussions with affected interests before any final report is issued. The principle that the cost of the main land-drainage system of an area should be financed by the community it serves has long been established and is not changed by the proposals of the Land Drainage Bill at present before Parliament. Therefore, as land-drainage authorities it is thought that the new conservation bodies should inherit from the river boards those boards' sources of income, that is, precepts on local authorities and on internal drainage boards, the proposed general and special drainage charges and the Exchequer grants payable to the boards at the present time.

As conservation authorities, expenditure would be incurred on administration and control, research, and on the building and maintenance of a variety of works to ensure the best use of the water resources. It is thought that this expenditure should be self-supporting on the basis of income received from water users within the area. The exact method by which this income would be raised has yet to be decided: licence fees, consumption charges, flat rates, etc., will be fully considered. There is, however, likely to be a special feature in the early years in that some major works will be desirable well in advance of the maximum demands which are likely to arise. Present-day users cannot always be expected to meet the full cost of works which will serve the interests of the new generation, and the Minister proposes that authorities should be empowered to levy a small precept (possibly not more than the product of a 1*d.* rate) for conservation works. In time it is thought that the water conservation authorities would be able to accumulate reserves against forthcoming capital expenditure and that the need for precepts would become less.

The financial suggestions are somewhat less definite than the hydrological proposals, and one can foresee a good deal of difficulty arising because of the inequality in the distribution of water resources, population and other economic resources. The intention not to seek powers to make Exchequer grants towards expenditure might well prove to be a pious hope. Some schemes are likely to be beyond the financial capabilities of the parent river basin and if completed might benefit larger areas. It would be desirable to consider the country as a whole as well as the individual parts. Clearly, much discussion and thought will be needed before the financial aspects are presented to Parliament.

The constitution of the water conservation authorities will also need careful thought. The principle underlying the constitution of the river boards is that those interests contributing to their revenues should be represented on them. This principle applied to the proposed conservation authorities would produce a large and unwieldy body. An alternative would be a small nominated group appointed by the Minister in consultation with the interests concerned. There is a constitutional difficulty here in that, apart from the Metropolitan Police, nominated bodies have not the power to precept. However, the water conservation authorities will themselves be unprecedented, and it may be that the interests concerned would be prepared to accept a nominated body. Another alternative would be a body half elected and half nominated: here the

Milford Haven Conservancy Act of 1958 might provide a guide.

It will be clear that some of the Minister's existing functions under the 1945 Act would with the above proposals pass to the water conservation authorities. The Minister's powers and responsibilities with regard to conservation would need re-defining in order to cover the co-ordination of the conservation authorities' assessments of local resources and potentialities; to watch from the national point of view the demand for water for domestic, industrial, agricultural and other purposes; to approve conservation schemes; to authorize and initiate schemes for inter-basin transference of water; to exercise appellate functions arising from decisions of the conservation authorities; and to approve capital investment programmes of the conservation authorities.

A new approach will also be necessary on the legal rights to particular water resources. Parliament has already considered it proper to restrict in some cases the right, arising from ownership of land, to take the water from beneath the land. It is further intended to amend the law so as to restrict the amount of water which can be taken without licence from boreholes sunk before control was applied, as well as to extend control to all underground waters. In the case of right to surface water, more particularly the rights of a riparian owner to use water flowing in a stream, it is proposed to provide that such an owner will be able to obtain as a right, from the conservation authority, a licence entitling him to the ordinary use of the water flowing past his land. "Ordinary" use will not include abstraction for irrigation and industrial purposes, and these uses will involve separate licences.

With water-storage capacity in Britain falling steadily behind rising consumption-rates, insurance against drought diminishes year by year and the need for both action and speed is pressing. The proposals outlined above are a welcome indication that the central Government authorities are at last alive to the necessity for comprehensive measures.

[1] Balchin, W. G. V., *Geog. J.*, **124**, Pt. 4 (December 1958).
[2] See *Nature*, **189**, 278 (1961).
[3] See *Nature*, **184**, B.A. 18 (1959).
[4] See *Nature*, **191**, 135 (1961).
[5] "Hydrological Activity in the United Kingdom", The Royal Society, London (1960).
[6] See *Nature*, **187**, 562 (1960); and p. 444 of this issue.

ACHIEVEMENTS IN PHYSICS

The New Age in Physics

By Prof. Sir Harrie Massey. Pp. 342+12 plates. (London : Elek Books, Ltd., 1960.) 42*s*. net.

ABOUT thirty years ago, physical science seemed to many of its students to have reached a peak of perfection. The nuclear atom held an established position and the power of quantum theory in interpreting atomic phenomena had been abundantly confirmed. Observational astronomy and thermodynamics had combined to predict the probable fate of the Sun and stars; only details apparently remained to be fitted into what was considered to be an elegant and satisfactory picture of the physical universe. One of the marks of this stage of achievement was the appearance of books of broad coverage, notably those of Jeans and Eddington, which sought to stimulate the interest of the general reader in the processes of scientific thought and experiment. The significance of these books was not diminished by the fact that the apparent self-sufficiency of physics in 1930 rapidly proved to be illusory. The spectacular discoveries in nuclear physics in the following decade became, as a result of awakening interest, increasingly matters of public concern.

By the end of the Second World War, physics was accepted as one of the basic elements of a scientific civilization, and it is now a commonplace that some knowledge of the subject and of its applications in contemporary affairs should form part of general education. Even more than in the days of Jeans and Eddington, there is a need for sober and balanced, but easily readable, works of exposition. It is to this need that Sir Harrie Massey's book makes a notable contribution.

The New Age in Physics is a book for the educated layman, but it can be read with pleasure and profit by the professional physicist. The mathematics used is of the simplest kind, but is sufficient to show that physics is above all a quantitative subject. Most of the book is devoted to atomic, nuclear and high-energy physics, and this is right, since it is on the atomic and nuclear scale that the new laws of physics exhibit their strangest manifestations. Sir Harrie Massey is known to the general public as an enthusiastic supporter of the space research programme, and it is therefore not surprising to find his chapter on neutrino phenomena ending with an account of hydrogen burning in the Sun, and his next (and final) three chapters devoted to radio astronomy, exploring the upper atmosphere, and artificial satellites and space probes. These chapters, like that on fission and fusion, are admirably lucid and compact accounts of the developments in physics which have most deeply excited the popular imagination. They will probably be more fully appreciated by the general reader than the rest of the book, which is concerned as much with concepts as with facts. Few non-scientific readers, however much they enjoy the adventures of Alice in "Looking-Glass House", will really find themselves greatly moved to learn that mirror symmetry is violated in weak interactions. Nor is the polarization of the vacuum a matter of everyday experience, although the ideas of special relativity are being increasingly discussed as interplanetary travel enters more within the bounds of possibility.

In his introduction the author sets out his intention to "choose a number of outstanding theoretical and experimental developments which illustrate the remarkable position which has been reached and the extraordinary rate of progress". In the event, one may comment that little choice has been necessary, since practically everything of general interest in atomic and nuclear physics, in the physics of the solid state, and in that of the solar system receives some mention. It is a pleasure to have authoritative and informative accounts of the contents of such an extensive field, and to sense an underlying unity of treatment which is often lacking in works of similar scope. The book gains much from well-chosen illustrations, and suffers but little from the finite number of misprints and the occasional errors in dates. It has an air of currency, but even so, it has just missed the Mössbauer effect, and it would be entirely consistent with the author's belief in the inexhaustible bounty

of Nature if some outstanding event were to occur before the book reached a second edition. Perhaps one may hope that when the stage is set and the space station exists, mankind may be offered the spectacle of another supernova in his own galaxy.

W. E. Burcham

A NEW APPROACH TO GENERAL RELATIVITY

Relativity

The General Theory. By J. L. Synge. (Series in Physics.) Pp. xv+505. (Amsterdam: North-Holland Publishing Company; New York: Interscience Publishers, Inc., 1960.) 110*s*.

THIS massive treatise is no mere conventional text-book of Einstein's theory of gravitation. On the contrary, although by a master of that subject, it is a highly individual work. For, although the author claims that the book is conservative in so far as it deals mainly with Einstein's theory in its original form, leading up to the famous triad of empirical tests, the mathematical treatment is novel. This treatment derives from Synge's conviction that, great as was Einstein's achievement, the true revolution in mental perspective was due to Minkowski, although this has not yet been fully appreciated by most students of the subject.

In accordance with a recent tendency among some students of relativity, Synge will have nothing to do with the principle of equivalence. Instead, he maintains that in Einstein's theory the existence of a gravitational field depends entirely on whether or not the curvature tensor does not or does vanish. This is an absolute property of space-time and does not depend on the observer. Synge accordingly endorses Minkowski's protest against the use of the word 'relativity' to describe a theory based on an 'absolute' (space-time). Indeed, he remarks that if Minkowski had lived to see the general theory of relativity he would have repeated his protest in stronger terms.

Although Synge's outlook is geometrical, in the sense that it is based on a thorough-going acceptance of the space-time way of looking at things with its fixed diagrams which are "easier to deal with" than the kinematical pictures of Newtonian mechanics, the peculiarity of his approach lies in combining this point of view with a deep conviction that time is the only basic measure, distance being essentially a derived concept. Consequently, Synge's version of general relativity is based on what he prefers to call Riemannian 'chronometry' rather than 'geometry'. This leads him to make general relativity more 'operational' (in Bridgman's sense) and thus more closely related to special relativity than has previously been customary. In this respect, I believe that Synge has been indirectly influenced by the pioneer example of kinematic relativity.

In considering the geodesic world-line of a particle in free fall, Synge interprets gravitational acceleration as a consequence of the curvature of the world-line of the observer. He is thus led to develop a new method of gravitational analysis based on a function first introduced in 1931 by H. S. Ruse. This function, which Synge calls the 'world-function,' is, to within a trivial factor, the square of the geodesic distance between two events in space-time, regarded as a function of the eight co-ordinates concerned. His use of this function has the advantage of combining the power-series technique with that of the tensor calculus.

Among the many topics discussed are Fermi transport, the material continuum and field equations, integral conservation laws and equations of motion, special universes, gravitational waves and geometrical optics. There is an extensive bibliography with references to reviews. The print is clear and the formal mathematics is relieved from time to time by the author's irony and wit. Irrespective of whether one accepts his point of view, his book will be found original and stimulating.

G. J. Whitrow

NEW HANDBOOKS FOR LUNAR STUDIES

Structure of the Moon's Surface

By Dr. Gilbert Fielder. Pp. xiii+266. (London and New York: Pergamon Press, 1961.) 50*s*. net.

Surface of the Moon

Its Structure and Origin. By V. A. Firsoff. Pp. 128+10 plates. (London: Hutchinson and Co. (Publishers), Ltd., 1961.) 21*s*. net.

Orthographic Atlas of the Moon

Compiled by D. W. G. Arthur and E. A. Whitaker. Edited by Gerard P. Kuiper. Edition "A" showing the Standard Orthographic Coordinate Grid. Part 1: Central Area. (Contributions, Lunar and Planetary Laboratory, No. 1.) Pp. 3+31 plates. (Tucson: University of Arizona Press, 1960.) n.p.

RESEARCH into the nature of the Moon's surface has become increasingly intensive in recent years. This increase can partly be ascribed to the advances in 'space-science', in particular the Russian achievement in photographing the Moon's 'other face'. However, new ideas had begun to appear before this. Spurr's *Geology Applied to Selenogy* was published between 1944 and 1949; although neither Dr. Fielder nor Mr. Firsoff subscribe to Spurr's speculative theories, they both believe that terrestrial geology is of prime importance in interpreting the appearance of the Moon's surface. They also share the view that the morphology of the surface features is a more rewarding line of research than the recording of ever smaller minutiæ.

Dr. Fielder's book is a complete handbook to the Moon. It is divided into two parts: the first describes the methods used to determine the physical properties of the Moon's surface and discusses the available observations. The second part is devoted to the morphology of the lunar surface, where the author's own researches play a more prominent part. Ray systems, the grid system, rilles and wrinkle ridges, and the distribution and frequency of craters are subjects well fitted to this approach. The book concludes with a chapter on the origin of the Moon's surface. This does not support any particular theory but is confined to what may reasonably be inferred from observation. Dr. Fielder has covered his subject completely and thoroughly—the only faults are occasional lapses into irrelevance.

For many years there have been two rival theories for the formation of lunar craters—the meteoric, which explains them as the result of meteors striking the surface, and the volcanic, which identifies them with extinct volcanoes. Mr. Firsoff's book is

an exposition of his own theory, which is derived from the latter. The craters are now identified as collapsed volcanic calderas, the result of the collapse of the surface layers after the magma beneath them had been spewed out in a volcanic eruption. The book is divided into three parts of which the first outlines what is known about the Moon. The second examines the inadequacies of the meteoric theory and puts forward the 'caldera' theory. The third is concerned with reducing our present knowledge of the Moon's surface to a coherent system; it is largely devoted to an examination of the analogies between the surfaces of the Moon and Earth. The chief criticism to be levelled at this clear and closely reasoned book is the reckless use of the word 'random'. In a scientific work this word should be confined to its statistical sense.

In 1960 a team of workers led by G. P. Kuiper published their *Photographic Lunar Atlas*. This covered every part of the Moon's surface four times, at differing angles of illumination, on a scale of one millimetre to every 1·37 km. on the Moon. The group have now published the first of a series of supplements. Twenty-nine of the best prints in the *Atlas* have been chosen to give a single coverage of the central portion of the Moon's disk. These are over-printed with a grid showing the orthographic ξ,η co-ordinates, in other words, rectangular co-ordinates fixed in the Moon with ξ axis northwards and ζ axis towards the Earth. The side of each square is 0·01 of the Moon's radius and positions can easily be read to an accuracy of 0·001. There are two editions: (*A*), on paper as described above and (*B*), on 'Tensalex' plastic, with an additional grid showing selenocentric longitude and latitude at 2° intervals.

D. H. P. Jones

ISSUES IN EVOLUTION

Evolution after Darwin

Edited by Sol Tax and Charles Callender. Vol. 3: Issues in Evolution. Pp. viii + 310. (Chicago: University of Chicago Press; London: Cambridge University Press, 1960.) 60*s*. net.

THIS is the third volume of the Darwinian Centennial Symposium held at Chicago in 1959 (see *Nature*, **189**, 340; 1961 for review of previous volumes). It comprises a first section on "Evolution and Religion" presented as essays on "Creation and Evolution in the Far East" by Ilza Veith, "Current Roman Catholic Thought on Evolution" by J. Franklin Ewing, *S. J.*, and "Creation and Causality in the History of Christian Thought" by Jaroslav Pelikan (it is refreshing to note that a Roman Catholic view has been included). The second section is given over to the centennial celebrations, made up chiefly of a television preview panel of five scientists and including Adlai Stevenson, five panels on the origin of life, the evolution of life, man as an organism, the evolution of mind, and social and cultural evolution, with a variety of chairmen and panellists. There is a convocation address by Sir Julian Huxley, a final television postscript panel and a personal conclusion by the general editor, Sol Tax. The index to the three volumes is included in this third volume, and there are also lists and photographs of the participants and panels.

The five panels were essentially a form of glorified 'brains trust' on a high level, and they were all given in public to audiences of about a thousand people. Members of the panels for the most part were generally those who had previously given papers in the first two volumes of this series. Thus the opportunity was given to tie up loose ends, to give the participants a chance to air their agreements and disagreements, and to put forward their ideas. These panels were most interesting, and probably their chief result will be to enable readers of the whole series of essays to sort out facts from speculations and to assess the present state of evolutionary ideas.

It is perhaps significant that the three essays on "Evolution and Religion" together amounted to only 40 pages, less than half the number of pages of some of the essays on specialized topics in the first two volumes. About 90 pages were given to such subjects as the "Origin of Life"—a subject which surely by any serious standard is about 99 per cent speculation and fancy and only 1 per cent fact. The contrast here is illuminating, but, dare I say it without fear of excommunication by my fellow biologists, it also pinpoints the paucity of evolutionary theory as a whole in spite of the brilliant expositions of its magnificent edifice by so many scientists; it also shows the weakness and barrenness of a purely materialistic concept of evolution and indicates without doubt that modern evolutionary theory has only a limited application to human beings. Why must biologists shut their eyes to the spiritual in man, pretend it is not there and thus remain only on the fringes of the study of the nature of man, who is demonstrably infinitely more than a mere animal? It is a tragedy that a wonderful chance to produce a modern synthesis of knowledge of man and evolution has been missed in this great and superbly produced series of books born of a great idea by Sol Tax. But still the three books as a whole stand as probably the best of their kind so far published, and the committee under Sol Tax, the contributors and the University of Chicago, which made it possible, are to be congratulated and thanked.

P. G. Fothergill

HETEROCYCLIC CHEMISTRY

Pyridine and Its Derivatives

Edited by Erwin Klingsberg. (The Chemistry of Heterocyclic Compounds: a Series of Monographs, Vol. 14). Part 1. Pp. x + 613. 367*s*. Part 2. Pp. x + 576. 282*s*. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960 and 1961.)

Heterocyclic Systems with Bridgehead Nitrogen Atoms

Part 1. By William L. Mosby. (The Chemistry of Heterocyclic Compounds: a Series of Monographs, Vol. 15). Pp. viii + 747. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1961.) 360*s*.

WITH two or three exceptions, previous volumes in the Interscience Series, *The Chemistry of Heterocyclic Compounds*, have tended to dwell on the less common heterocyclic systems. This is true of only one of the three volumes under review, and it is interesting to see the treatment accorded to a more familiar topic—*Pyridine and its Derivatives*. The first two volumes discussed here constitute Parts 1 and 2 of a four-volume treatise, which will surely

become the standard reference work on pyridine chemistry and a worthy successor to Maier-Bode's *Das Pyridin*.

Volume 1 comprises two chapters, the first of which is devoted to an introductory account of the physical properties and chemical reactions of pyridine and its simple derivatives. This is, in itself, a useful 90-page summary of pyridine chemistry and the author has made an admirable job of correlating and unifying the subject in terms of electronic theory. He has also provided an appropriate introduction to the remaining volumes of the treatise which deal, in detail, with a number of specialized topics. The remaining chapter of the first volume is entitled "Synthetic and Natural Sources of the Pyridine Ring", and is both comprehensive (500 pp.), and systematic. Every known synthetic method appears to have been included, and several intriguing, little-known reactions are brought to light. (Is benzene really transformed into pyridine by carbonyl azide ?) Also included is an interesting table of pyridine derivatives which have been encountered in alkaloid degradation reactions. A feature of this chapter is the inclusion of 240 pages of tables, illustrating the scope of each synthetic method in a style reminiscent of *Organic Reactions*. Mechanisms are mentioned only briefly, and Chapter 2 will be valued chiefly as an exhaustive survey of synthetic methods, for as such it is a notable contribution to organic chemistry.

In Volume 2, the detailed discussion of special topics begins, and within its six chapters this volume covers organo-metallic compounds, quaternary compounds and N-oxides, alkyl, aryl, halo- and nitro-pyridines. Each type is discussed systematically under the sub-headings, preparation, properties and reactions, and there is liberal provision of tables at the end of each chapter; these summarize reaction conditions and products, or alternatively list physical constants. Spectral data are rather scarce, and no curves are reproduced. Likewise, little attention is paid to reaction mechanisms; for example, the chemistry of pyridine-N-oxide is described (30 pp.) with the use of only three curved arrows. In fairness it must be admitted that by following the references provided, the reader will be able to find the spectra he is seeking, and will encounter a more mechanistic treatment of the subject. The editor may have preferred to stick to facts, and for a series which is destined to endure for several decades, this is probably a wise decision. Nothing dates a book more than out-moded theory.

The third volume discussed here is entitled *Heterocyclic Systems with Bridgehead Nitrogen Atoms*, Part I. This deals only with fused ring-systems and begins with the smaller rings. (A second volume is planned to complete the discussion of fused systems and to deal with bridged systems.) This is a fascinating book, which assembles and classifies a group of heterocycles, many of which have never before been reviewed. However, it is no mere collection of curiosities. Many of the claims in the literature reported in this book are highly improbable, and the author has been commendably critical in discussing them. The earlier chapters, in particular, are rich in unsolved problems. Later chapters deal with less-obscure topics (for example, pyrrocolines) and provide tables of derivatives. An exhaustive review of penicillin chemistry would have been impracticable and the author has elected to describe only syntheses of the thiazolidine-β-lactam system, and to provide key references.

This volume will probably attract less attention than those mentioned above, but the author is to be congratulated on his treatment of a difficult subject; this is a valuable contribution to the chemical literature.

G. L. Buchanan

COASTAL INLETS

Stability of Coastal Inlets

By P. Brunn and F. Gerritsen. Pp. xvii + 123. (Amsterdam: North-Holland Publishing Company, 1960.) 23*s*.

THIS book is concerned with the stability of channels connecting estuaries and inlets with the open sea. The depths in such channels are becoming increasingly critical as the dimensions of ships are enlarged; whereas 30 ft. was a generally acceptable depth fifty years ago, depths of up to 50 ft. are required to accommodate the vessels of 100,000 tons now being built. As a result many estuaries in which important ports are situated are being closely examined—often with the aid of hydraulic models and theoretical studies—to find out if their depths can be increased and their navigational facilities improved. The estuaries of the Rivers Thames, Mersey and Tees are among those being investigated in Great Britain.

These investigations are much handicapped by the lack of sound theoretical foundations on which the design of stable channels in mobile material can be based, especially when the complications introduced by tidal flow and littoral drift have to be considered. By assembling an extensive collection of data on the régime in coastal inlets, and by developing the theoretical foundations on which calculations can be made, the authors of this book have made very valuable contributions to the solution of the complex problems involved in the design of coastal inlets, and in the improvement of existing estuarine channels. The authors are, respectively, head, and associate professor of the Coastal Engineering Laboratory of the University of Florida, and have very extensive experience in coastal investigation.

The book opens with some general remarks on the origin of coastal inlets; this is followed by a comprehensive review first of the theoretical relationships which have been developed from the dynamical and continuity equations describing tidal flow in inlets, and second, of the theories governing the stability of bed material—ranging from the empirical equations developed by Lacey (1930) to the rational theories of Lane (1958), Chien (1957) and others. The significant forces involved in inlet stability are then considered and a relationship between stability sheer stress, maximum tidal flow and cross-sectional area is given. An analysis of data taken from existing inlets follows, and graphs are given which show how the maximum tidal flows are related to cross-sections for a number of inlets and estuaries in Europe and the United States; it is demonstrated that the stability sheer stress has a general value of 0·080 lb./ft.^{2}, although particular inlets may have values ranging from about 0·072 lb./ft.^{2} (where littoral drift and sediment loads are low) to about 0·103 lb./ft.^{2} (where littoral drift and sediment loads are relatively high).

Later in the book the methods by which sand is passed across inlets are discussed, and it is suggested that there are two radically different methods of by-passing. If the ratio of predominant littoral

drift (cubic yd./yr.) to maximum spring tidal flow (cubic yd./sec.) is greater than 200–300, sand generally crosses the inlet on a submerged offshore bar: if the ratio is less than 10–20 the transfer of sand across the inlet involves the movement of tidal channels, and there is no stable offshore bar.

The book ends with a short section on the design of tidal inlets, and it is pointed out that, for stable inlet conditions, the tidal prism and the maximum tidal flow must be large in comparison with the littoral drift and that a suitable stability sheer stress must be chosen. A comprehensive bibliography is included.

Stability of Coastal Inlets can be recommended to all those interested in the design of new inlets, or the improvement of existing inlets and estuaries.

W. J. Reid

MATHEMATICAL STUDIES IN THE PSYCHOLOGY OF WAR

Statistics of Deadly Quarrels

By Dr. Lewis F. Richardson. Edited by Quincy Wright and C. C. Lienau. Pp. xlvi+373. (London: Stevens and Sons, Ltd., 1960.) 63*s*.

Arms and Insecurity

A Mathematical Study of the Causes and Origins of War. By Dr. Lewis F. Richardson. Edited by Nicolas Rashevsky and Ernesto Trucco. Pp. xxvi+307. (London: Stevens and Sons, Ltd., 1960.) 63*s*.

The Strategy of Conflict

By Prof. Thomas C. Schelling. Pp. vii+309. (Cambridge, Mass.; Harvard University Press; London: Oxford University Press, 1960.) 50*s*. net.

WARS begin in the minds of men. Hence the study of war is essentially a task for psychology, and as such demands scientific treatment. Specialists in other fields have been ready with explanations based on some cherished concept of their own—the instinct of pugnacity, the growth of population or the expansion of trade, the ambitions of nations or their leaders, a society based on acquisitiveness and aggression, carried away by emotional forces similar to those that generate conflict and disharmony in the mind of the individual. But, until quite recently, no one has specialized in research on war psychology as such, or attempted a systematic analysis of the available data to check and verify these alternative theories by the methods of empirical science.

The pioneer in this newer approach was Lewis Fry Richardson. A Quaker by upbringing and a physicist by training, his guiding principle was to study war and peace by the same quantitative techniques that he developed for the solution of apparently intractable problems in the field of physics; and as early as 1919 he published a short pamphlet on *The Mathematical Psychology of War*. His best-known work was in applied mathematics and meteorology. His highly original paper on "The Approximate Solution by Finite Differences of Physical Problems involving Differential Equations" anticipated the method of 'relaxation'; and his book *Weather Prediction by Numerical Process* has been acclaimed as "the first textbook on dynamic meteorology".

In 1926 he was elected a Fellow of the Royal Society; and shortly afterwards, at the age of forty-eight, took an honours degree in psychology. From 1930 until his death in 1953 he devoted himself almost entirely to what he called "generalized foreign politics". His various investigations were collected and embodied in two major books, which, thanks to the generosity of United States scholars and the Littauer Foundation, have now been edited and published.

The first is concerned primarily with the problems and consequences of an 'arms race'. Richardson's plan is to express the underlying tendencies in the form of two differential equations. From these he deduces a variety of corollaries which he then seeks to verify by comparing the theoretical 'predictions' with the actual data for the periods that ushered in the two World Wars. A further deduction, based on the usual method for determining a position of equilibrium, enables him to formulate the conditions that are most likely to ensure a stable balance of power.

His second book treats war as a "large scale example" of what he terms "deadly quarrels"—a phrase which includes any form of human conflict issuing in the death of one or more of the contestants —murder, gangsterism, and banditry, as well as the wars of history. With amazing industry he has compiled a mass of information relating to the causes of the various outbreaks during the years 1820–1929, and supplements it by the data collected by Prof. Quincy Wright for the "wars of modern civilization". He then attempts to estimate the relative importance of the causes commonly alleged by an exhaustive statistical analysis. His chief conclusion is that wars are due to a wide variety, and usually to a plurality, of predisposing and precipitating factors. The mistake of previous theorists was to single out and over-emphasize just one of many. Of the three main groups he considers geographical conditions more important than economic, and psychological more important than either.

Both volumes, however, are restricted to conditions obtaining before the Second World War, and are therefore of greater interest to the historian than to the student of contemporary politics. Dr. Schelling, who is professor of economics and an associate of the Centre for International Affairs at Harvard University, is concerned with newer problems arising from the development of nuclear bombs and intercontinental missiles. His method is to estimate probabilities rather than to measure causal forces; and his mathematical techniques are those of the economist rather than the physicist. He points out that the present situation is no longer one of "pure or absolute conflict", but of "conflict resting on a basis of certain common interests". On either side the policy that results is a series of competitive manœuvres in which each tries to win the maximum advantage for himself without necessarily inflicting an equal disadvantage on the other. In simplified form such situations are dealt with in the 'theory of games' where the game is of the 'non-zero sum' type. The conclusions to which his arguments lead are often unexpected, sometimes paradoxical, but always instructive. He warns us that the analysis he adopts assumes that both sides argue rationally, whereas in practice the behaviour of rival powers is largely influenced by a variety of irrational factors—personal, emotional, or even wholly accidental. This, however, presents problems in political psychology, varying from moment to moment, rather than in political arithmetic. The moral of it all is that, if you want peace, it is not enough to prepare for war, you must seek to understand it.

Cyril Burt

XUM

Magnetic Materials in the Electrical Industry

By P. R. Bardell. Second edition (revised). Pp. 320. (London: Macdonald and Co. (Publishers) Ltd., 1960.) 32*s*. 6*d*. net.

THE scope of this book is precisely indicated by its title: it is about magnetic materials, not about magnetism, and it describes their uses rather than, though not to the exclusion of, their general properties. There is a further restriction: the author's interests are mainly in communications and his book is biased accordingly. He is an engineer and his book is primarily for engineers, although it might also interest a physicist who wants to find out the technical relevance of some of the properties he measures. This prying physicist would occasionally raise his eyebrows; for example, at some diagrams of magnetic domain boundaries of types recognized since about 1950 as impossible; but one or two such slips do not affect the general practical usefulness of the book. The only serious lapse is that the author thinks that manganese-zinc and other double ferrites are used for the sake of increased permeability rather than for increased flux-density.

The text uses trade names freely, and for many readers this will be helpful. The book is generously illustrated, both with diagrams which are good and with photographs which do not really add much to the text. The contents pages and index are good. One gem of over-zealous editing is included: a 10^{11} in the manuscript was presumably rendered by the typist as 10" and now appears as 10 in. Fortunately this is not typical; the proof-reading is good and the book is well produced. It packs a great deal of information into a reasonable space and within its intended limited scope it is to be recommended.

A. C. Lynch

The Physiology of Crustacea

Edited by Talbot H. Waterman. Vol. 2: Sense Organs, Integration, and Behaviour. Pp. xiv + 681. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 23 dollars.

THIS volume completes the survey of physiological processes in Crustacea which was so well begun by the volume published last year. There are thirteen chapters written by separate authors dealing with a wide range of topics, but all related in some way to the means by which a crustacean receives information abouts its environment and the ways in which it responds to this information. The general standard of these chapters is excellent; the two middle chapters by Wiersma on the neuromuscular system and the central nervous system are particularly valuable. They provide a link between the chapters dealing with sensory perception and the chapters on locomotion and complex behaviour. The link is by no means complete yet, but the crustacean nervous system is most economically organized and contains relatively few neurons so that it offers the possibility of a type of analysis which would be much more difficult to achieve in vertebrates. The fourteenth chapter is an attempted synthesis of the two volumes, and might almost be described as a philosophers' guide to comparative physiology.

As in the first volume one is impressed by the amount already known about the physiology of the Malacostraca, and the Decapoda in particular, but one also gains a strong impression that the lower Crustacea are greatly in need of more detailed study. This is one of the great strengths of the book; the gaps in our knowledge are clearly shown.

In a work of this size it is inevitable that a few minor errors should creep in; *Limnadia*, for example, is not a notostracan as might be inferred from Table 1 in the first chapter, and the young stages of *Bosmina coregoni* would not generally be regarded as metanauplii. These errors do not detract from the great achievement of the editor in bringing together these excellent summaries. The information presented and the detailed bibliographies make this book essential to every student of the Crustacea, and provide the general zoologist with a detailed, up-to-date account of the nervous system and behaviour of this large group.

J. Green

Sound Production and Sound Reception by Insects

A Bibliography. By Mable Frings and Hubert Frings. Pp. vi + 108. (University Park, Pennsylvania: Pennsylvania State University Press, 1960.) 4 dollars.

THIS is a bibliography which will prove useful to the increasing number of students of sound production and hearing in insects. It includes 1,752 titles, and is tolerably complete up to the end of 1957. No annotations are provided but a simple set of symbols indicates the ground covered by each paper or book quoted. The bibliography proper is followed by two indexes which refer to the numbered entries in the bibliography; one is classified under the orders and families of insects, the other under the types of organ used for sound reception. The authors proceed on the assumption that any mechano-receptor may on occasion be brought into service for the detection of waves, vibrations or rapid barometric changes in the surrounding medium, and may serve as an occasional organ of hearing. The bibliography therefore covers a wide field. Modern methods of recording and analysing sounds have attracted more workers into this field in recent years: the present compilation should be widely used.

V. B. Wigglesworth

Doctors and Disease in Tudor Times

By W. S. C. Copeman. Pp. xiv + 186 + 13 plates. (London: Dawson's of Pall Mall, 1960.) 42*s*.

THIS book can be read with pleasure, profit and interest. It is also a book to have by you so that it can be read or referred to again and again.

It begins well with an introduction that is unusual because it is good; it is in itself a concise essay on the importance of, and justification for, presentation of medical history.

The first chapter is especially acceptable because it gives an excellent review of the history not only of medicine but also of Europe as well. The evaluation of the profession is thus presented in its relation to the whole pattern of life and society.

As a whole this book contains in a concise yet clear and readable form valuable information about the historical evaluation of medicine that makes one somewhat disturbed, even ashamed, that it is not generally completely known, so fundamental is it.

In some chapters a certain amount of repetition indicates the origin of the book in separate lectures. While this is noticeable when the book is read as a whole it would not be noticeable when it is used for reference, and in this way the repetition is in fact likely to be helpful.

The author is to be congratulated on such a scholarly, interesting and eminently readable book.

Russell Brock

SCIENCE IN THE HIMALAYA

By Dr. L. G. C. E. PUGH
National Institute for Medical Research, London

THE Himalayan Scientific and Mountaineering Expedition, 1960–61, led by Sir Edmund Hillary, and which was sponsored by the *World Book Encyclopædia*, recently concluded its operations after a period of nearly ten months in the field. The final stages were marred by an unfortunate series of accidents, due to illness, which led to the withdrawal of the party from its final mountaineering objective—the ascent of Mount Makalu without the use of supplementary oxygen. Nevertheless, the programme of scientific research, both on Mount Makalu and in the Ming Bo region, near Mount Everest, where the party spent the winter, was successfully completed.

The expedition set out from Katmandu at the beginning of September 1960, and returned during May–June 1961. Scientific work was carried on continuously from December to May on the Ming Bo Glacier at an elevation of approximately 19,000 ft. Physiological observations on a smaller scale were made on Mount Makalu at altitudes of 21,000 ft., 24,000 ft. and 26,000 ft.

The winter programme was made possible by the provision of a prefabricated hut designed for the expedition by E. Levin, of the Timber Development Association, Ltd., London. The hut provided complete immunity from the weather, reasonably stable indoor temperatures around 60°–70° F., and excellent lighting. It was a cylindrical structure composed of about 100 sections and weighing approximately 1 ton. The walls consisted of 4-in. thick plywood panels of stressed skin construction, lined with aluminium foil and filled with foamed plastic. Heating was provided by a kerosene stove, designed for operation at 20,000 ft., by the British Petroleum Co.'s Research Centre at Sunbury-on-Thames. Electricity for lighting and scientific apparatus, including a radio transmitter, was provided by a Lucas wind generator and a motor generator, with 24-V. nickel–iron storage batteries. The hut stood on jacks on a snow surface and was held down by steel cables.

The scientific team was organized and led by Dr. L. G. C. E. Pugh, of the Division of Human Physiology, National Institute for Medical Research, and was composed as follows: Mr. B. Bishop, National Geographic Society, Washington (glaciologist); Mr. M. G. Gill, University of Otago, Dunedin, New Zealand (medical student); Prof. S. Lahiri, Presidency College, Calcutta, 12 (physiologist); Dr. J. S. Milledge, University of Birmingham and the Southampton Chest Hospital, Southampton, Hants (physician); Captain T. Nevison, U.S. Air Force Medical School (physiologist); Dr. M. P. Ward, Department of Surgery, London Hospital, Whitechapel, London, E.1 (surgeon); Dr. J. B. West, Department of Medicine, Postgraduate Medical School, Hammersmith Hospital, London, W.12 (physiologist).

Captain B. Motwani, of the Defence Science Laboratory, Delhi, was attached to the Expedition as an observer, and assisted with physiological and other work.

The only non-scientist with the party was W. Romanes, a builder from New Zealand. His knowledge and skill played an important part in the successful erection and maintenance of the laboratory hut and its equipment. He also acted as assistant to B. Bishop in his glaciological and meteorological work.

As may be inferred from the composition of the team, the emphasis in planning the scientific programme had been placed on physiological research. Studies in glaciology and meteorology were also undertaken, but on a more limited scale. A programme of psychometric testing designed by the Medical Research Council's Applied Psychology Unit at Cambridge was carried out by M. Gill, as well as tests of the special senses of hearing and vision.

The principal physiological investigations carried out during the winter were as follows:

(1) *Functions of the heart.* The cardiac output was measured by the acetylene technique in men exercising at three work intensities on a bicycle ergometer. Serial electrocardiograms were taken at intervals throughout the expedition.

(2) *Pulmonary function.* The diffusing capacity of the lungs for carbon monoxide and oxygen was measured at three grades of muscular exercise.

(3) *Respiratory regulation.* The response threshold and sensitivity of the respiratory centres to carbon dioxide and the interaction with oxygen were studied, using a technique worked out by Lloyd and Cunningham, and their associates in the Department of Physiology at the University of Oxford.

(4) *Respiratory exchanges.* Oxygen-consumption, lung-ventilation and heart-rate were measured under basal conditions and during muscular work at 300, 600 and 900 kgm./min. on the bicycle ergometer. Data were obtained on the effect of breathing supplementary oxygen.

(5) *Blood.* Samples of arterialized venous blood from the heated hand at rest and during exercise at three rates of work were analysed for oxygen and carbon dioxide. The arterial oxygen saturation at rest and exercise was also followed, using a Waters Conley ear oximeter. The effect of a prolonged sojourn at altitude on blood volume, hæmatocrit, red cell and hæmoglobin concentration was studied.

(6) *Renal function.* The following tests were carried out: maximum and minimum *p*H of urine after suitable doses of sodium bicarbonate and ammonium chloride, respectively; the maximum specific gravity during water deprivation; maximum water elimination after a standard water load.

On Mount Makalu (27,900 ft.) the respiratory exchanges were measured on subjects working on the bicycle ergometer at 21,000 ft. and 24,000 ft. and electrocardiograms were recorded at both of these camps. Twenty-five samples of alveolar air were taken at 26,000 ft. and brought back to London for analysis.

Results

Preliminary results of some of these investigations are already available, having been worked out before

leaving the Himalaya. The principal findings were as follows:

The electrocardiographic records obtained at 19,000 ft. showed marked right axis deviation, which was not abolished by breathing oxygen. The output of the heart for a given work-load on the bicycle ergometer was somewhat lower than the control values at sea-level; the maximum output was limited to 16–18 l./min. compared with 25–30 l./min. at sea-level. Heart-rates at low work intensity were higher than sea-level control values at the same work-rate, but during maximum work the heart-rate was comparable with or lower than the corresponding sea-level value. Maximum heart-rates were limited to 130–150 beats/min. compared with 180–190 beats/min. at sea-level.

The diffusing capacity of the lungs for carbon monoxide and oxygen during exercise was not significantly altered by prolonged residence at altitude, the results being closely comparable with control values obtained at sea-level. Arterial blood-oxygen saturations of 65–70 per cent were observed in resting subjects at 19,000 ft. During severe muscular exercise arterial oxygen saturations fell to 50–55 per cent—values which would not be consistent with consciousness in unacclimatized subjects acutely exposed to lack of oxygen.

It was found possible to work for up to 5 min. at 900 kgm./min. on the bicycle ergometer at 19,000 ft. and 1,200 kgm./min. could be kept up for 2 min. by the fittest subjects. These work intensities were very much higher than those studied by previous investigators. Maximum oxygen intake during exercise at 19,000 ft. was 2·0–2·5 l./min. compared with 3·5–4·0 l./min. at sea-level when the subjects were less fit. Ventilation volumes of 120–150 l./min. body temperature, pressure, saturated were observed during sub-maximal work (900 kgm./min.), but at 1,200 kgm./min. values as high as 200 l./min. were recorded just before the breaking point. These results confirmed previous observations made on Mount Everest in 1953.

The studies of respiratory regulation indicated that the increased breathing at altitude is accounted for partly by a lowering of the threshold of response to carbon dioxide and partly by an increase in sensitivity to carbon dioxide. The interaction between oxygen and carbon dioxide, whereby carbon dioxide sensitivity increases at low oxygen tension, was not apparently affected by acclimatization to altitude.

Blood-volumes measured by the carbon monoxide method showed a small but significant reduction after 4–6 weeks at altitude; after 4–6 months, however, four out of six subjects studied showed major increases in blood-volume amounting to about 1 litre. The remaining two subjects showed no late increase in blood volume, and their hæmoglobin concentration and hæmatocrit values declined. It was thought that this might be associated with high-altitude deterioration.

Meteorology

Fine weather with clear skies prevailed throughout the autumn and winter. Apart from afternoon mist and a few short storms, the fine weather continued until the end of April, when the pre-monsoon period of unsettled weather set in.

The lowest temperature recorded was −27° C. Winds of 45 knots gusting to 60 knots were common during the winter, and it was seldom necessary for the motor generator to be used. In April, calm weather prevailed.

These observations, which are the first to be made at high altitude during the Himalayan winter, suggest that a safer time for climbing operations might be autumn or early spring rather than the traditional pre-monsoon season.

THE CHESTER BEATTY RESEARCH INSTITUTE (OF THE INSTITUTE OF CANCER RESEARCH: ROYAL CANCER HOSPITAL)

By Prof. A. HADDOW, F.R.S.
Director

AN extension of the Chester Beatty Research Institute (see Fig. 1) was opened by Sir Howard Florey, president of the Royal Society, on June 9, in the jubilee year of the formal inauguration of the Research Institute of the then Cancer Hospital (Free), by H.R.H. the Duke of Connaught on May 23, 1911. The main cost of the new construction was defrayed from funds contributed in the past by the charitable public, approximately £250,000 having been made available to the Committee of Management of the Institute of Cancer Research by the Board of Governors of the Royal Marsden Hospital, the latter acting as custodians. In addition, a grant of £40,000 was made by the Wellcome Trust, in recognition whereof a section of the extension has been designated the Wellcome Laboratory, for research in pharmacology and experimental chemotherapy. The new building, of which the architects were Messrs. Lanchester and Lodge and the contractors Messrs. Humphreys, is linked on four floors with the older Chester Beatty Research Institute, and contains extensive animal-house accommodation, laboratories in biology, organic chemistry, biochemistry, physical chemistry, immunology and cytogenetics, and a conference hall to seat approximately 180. The opportunity has also been taken to re-model the older building, and, among other things, to provide increased library facilities. The present scientific staff at the Fulham Road site, together with an experimental station at Pollards Wood, Chalfont St. Giles, Buckinghamshire (directed towards work on biological mutation, tracer biochemistry, nucleic acid chemistry, protein synthesis and virology), numbers about one hundred, with an approximately equal number of technicians.

It may be of interest to summarize the history of the institution over the past fifty years. The Cancer Hospital (Free), of which the Institute was to become

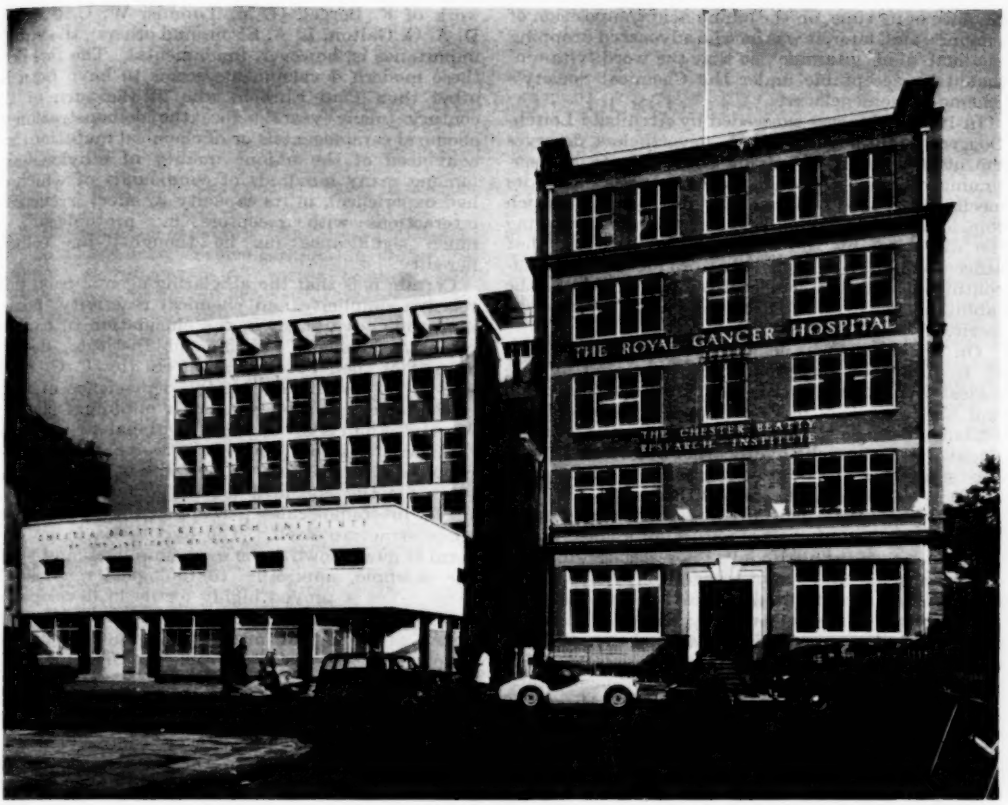


Fig. 1. The Chester Beatty Research Institute and the New Extension

a part, was founded by Dr. William Marsden in the year of the Great Exhibition. At St. Bartholomew's Hospital, Marsden was among the last students of John Abernethy, who had been surgical dresser to Sir Percivall Pott, in turn author in the latter part of the eighteenth century of a celebrated description of cancer of the skin as an occupational disease of chimney sweeps (due to contamination of the skin with soot)—a key observation in direct line with the great advances made by Kennaway and his school, at the Institute, 150 years later.

From its foundation, the Cancer Hospital soon achieved a considerable tradition of clinical research and discovery. However, at the turn of the nineteenth century, increasing recognition of the need for the experimental method led in due course to the establishment of a Research Institute, in 1909. Almost thirty years later its facilities proving inadequate, the Institute was transferred to new quarters in 1939, through the benevolence and vision of Sir Chester Beatty, in recognition of which the Institute was re-named. In the meantime, the Institute, with parts of the Physics and Radiotherapy Departments of the Hospital, had for several years been recognized as a School of the University of London.

With the advent of the National Health Service in 1948, the Institute became the nominal responsibility of the Ministry of Health. Very soon, however, mutual arrangements were made whereby the main financial responsibility was assumed by the Medical Research Council, and the Institute became affiliated to the British Postgraduate Medical Federation. Very necessary additional finance is provided by the British Empire Cancer Campaign, which has supported the Institute increasingly over the past thirty-eight years, and also on a substantial scale from the United States Government through its Public Health Service, and to a lesser, but valued, extent from private American benefactions such as the Jane Coffin Childs Memorial Fund for Medical Research, and the Anna Fuller Fund, of New Haven and Yale.

The first director was Alexander Paine, most noted for his work with Poynton on *Streptococcus rheumaticus*. In its earliest days the Institute rapidly attracted an extraordinary group of men—including Charles Singer, Schryver (its first physiological chemist), Keith Cannan (now of the National Research Council in Washington), Casimir Funk, Jack Drummond, G. W. Nicholson and E. H. Kettle. Casimer Funk, who at that time was studying the etiology of the deficiency diseases (beriberi, polyneuritis, epidemic dropsy, scurvy and pellagra), had coined the name 'vitamine' in 1912 (while at the Lister Institute), and joined the staff in 1913. Drummond himself joined Funk in 1914, and was appointed to succeed him as physiological chemist in 1918. During these years Drummond carried out much remarkable work, in

advance of its time, on the amino-acid composition of tumours, and, later, it was he who advocated dropping the final 'e' of 'vitamine', so that the word 'vitamin' should be acceptable under the Chemical Society's scheme of nomenclature.

In 1920, Paine was succeeded by Archibald Leitch. Dogged by ill-health, Leitch nevertheless deserves the utmost credit for giving the Institute its continuing theme—chemical carcinogenesis and its mechanisms—and, during his directorship, which coincided with a period of great stimulus following the discovery by the Japanese, in 1915–18, that cancer could be artificially produced by long-continued application of coal-tar to the skin of the rabbit or mouse, wrote several papers of classical merit.

On Leitch's death, he was succeeded in 1931 by E. L. Kennaway, who had been appointed physiological chemist ten years before. In the 1920's, Bloch and his co-workers in Zurich had already adduced evidence to suggest that the active agent in coal-tar might well be a cyclic hydrocarbon. Of this, Kennaway supplied a virtual proof, through the generation of artificial carcinogenic tars, either by passing isoprene or acetylene with hydrogen through heated tubes, or by the pyrolysis of natural products. Study by Mayneord of the ultra-violet fluorescence properties of carcinogenic tars led to recognition of a (largely) characteristic spectrum, and this essential clue in turn resulted in the detection by Hieger of the same spectral qualities in 1 : 2-benzanthracene, the discovery of the first chemical individual to manifest pronounced carcinogenicity (1 : 2-5 : 6-dibenzanthracene), the isolation by Cook, Hieger and Hewett in 1933 of 3 : 4-benzpyrene from carcinogenic pitch, and the generation (following a prediction by J. W. Cook) of the potent carcinogen 20-methylcholanthrene from deoxycholic acid.

In these earlier days it seemed possible that the cyclic aromatic hydrocarbons might exercise some specific function in carcinogenesis, and it is still not to be excluded that, by their formation in even trace amounts through local abnormalities of steroid metabolism, they could play an important part in the natural causation of cancer in man. However, the experimental findings of the past thirty years have shown beyond question that carcinogenicity is equally a property of many other chemical types, among which may be included various azonaphthalenes studied by Kennaway himself, a series of aminostilbenes described by Haddow, Harris, Ross and George Kon, many instances of endocrine carcinogenesis in the work of Harold Burrows and Eric Horning, and a range of polymers, plastics and metals—to name but a few.

In the past fifteen years, attention has naturally become centred on problems of the mechanisms of action. The mode of action of the hydrocarbons themselves and of the aromatic amines is still obscure, although valuable progress has been made through the investigations by Boyland and his group of the intermediary metabolism of such agents. It is probably true to say that of all the groups of carcinogenic agent more recently recognized, the now very large class of so-called biological alkylating agents is the most important. Of some practical relevance in that they have yielded chemotherapeutic agents such as 'Myleran', 'Chlorambucil' and 'Melphalan' of useful application in the treatment of chronic myelogenous leukæmia, chronic lymphatic leukæmia and myelomatosis respectively (from the work of F. Bergel, G. M. Timmis, W. C. J. Ross, D. A. G. Galton, L. A. Elson, and others), their main importance is, however, fundamental. The father of these modern developments seems to have been no other than Paul Ehrlich, who at the turn of the century (many years before the demonstration of chemical carcinogenesis or of chemical mutation) was convinced of the unique quality of ethyleneimine (among many hundreds of compounds of which he had experience), in its capacity to effect irreversible interactions with receptors in 'protoplasm', of much significance (as he thought) for cellular heredity.

Certain it is that the alkylating agents, from their relative simplicity and chemical reactivity, possess numerous advantages in the elucidation of carcinogenic mechanisms. Within the Institute, detailed studies of their cytological effects (by P. C. Koller and S. H. Revell), and of the dependence of many of the biological properties on a minimum chemical reactivity and a bi- or poly-functional structure, led Goldacre, Loveless and Ross twelve years ago to propose the cross-linkage hypothesis, with implications for the chemical cross-linkage of contiguous linear macromolecules perhaps within the chromosome structure itself. The hypothesis in its early form is now known to be over-simplified, and is not, for example, applicable to biological mutation at large. Yet it proved highly fertile in development, and rapidly led to recognition of new alkylating types of biological interest, a process in which much help was gained from the field of textile technology.

A key problem concerns the relative importance of the reactive groups in proteins, nucleic acids and nucleoproteins, which may be the centres of attack—with special reference to the striking dethiolating properties of 'Myleran' studied by J. J. Roberts and G. P. Warwick, to the esterification of phosphate groups brought about by other alkylating agents (P. Alexander, W. C. J. Ross), and to alkylation of the purine and pyrimidine bases. Involvement of purines was reported by Press and Butler some years ago, and later work by Lawley showed the special importance of *N*-alkylation at the 7-position of guanine. Of special interest is the possibility that esterification of phosphate groups, and alkylation of purine moieties, may represent consecutive steps in a reaction leading to delicate interference with the integrity of the nucleic acid structure. Most recently, Lawley and Brookes have recovered, among other products of the reaction of both sulphur and nitrogen mustards, *bis*-guanyl structures which very likely—although there is no formal proof—reflect cross-linking of the twin spirals of the Crick–Watson helix.

In general, the work of the Institute points towards a conclusion identical with that being reached by many other laboratories using different lines of attack, namely, that malignant transformation may involve biochemical deletion, with permanent release from those enzymic systems responsible for homeostasis of the growth potential of the normal cell. Diverse as are not only chemical but also physical and viral carcinogens, in their nature and modes of operation, and indeed in the tumours they produce, it is still not to be excluded that they may, by varied routes, lead to a key alteration similar or even identical in the various cases; and it is towards some such synthesis that the work of the Institute will continue to be directed.

METABOLIC PRODUCTION OF SUCROSE FROM FAT

By Prof. H. BEEVERS

Department of Biological Sciences, Purdue University, Lafayette, Indiana

THE production of sucrose from reserve fat occurs on a large scale in some germinating seeds. For example, an ungerminated castor bean contains some 260 mgm. of fat (70 per cent of its dry weight) and 15 mgm. of total carbohydrates[1]. During germination at 25° C. in the dark over a period of 8 days the total fat content of the seedling falls to about 50 mgm. while the carbohydrate rises to 230 mgm., and this is in spite of the fact that sugars are being used extensively in the growth and respiration of the seedling proper. Thus there is a production of more than 1 gm. of sugar (principally sucrose) for each gram of fat consumed. This interconversion is strictly confined to the endosperm tissue (respiratory quotient (*RQ*) about 0·35); sucrose moves into the growing seedling where it supports carbohydrate respiration (*RQ* = 1·0).

In considering the pathway of this interconversion, we should note first that glycerol is converted quantitatively into sucrose in the endosperm and quite efficiently in the cotyledons also[2]. The labelling pattern in the hexose produced from glycerol-1,3-^{14}C shows that the intact 3-carbon unit is incorporated, presumably after conversion to triose[2]. It should be clear, however, that the synthesis of sucrose from the glycerol moiety of the fat could account for only a small fraction of the conversion actually observed. Thus the problem resolves into the synthesis of sucrose from long-chain fatty acids.

The metabolism of fatty acids in plant tissues has been intensively investigated by Stumpf *et al.*[3]. Although enzymic mechanisms inducing α-oxidation engaged earlier attention, the metabolism of specifically labelled acids by tissue-slices pointed to the operation of a β-oxidation pathway *in vivo*, and such a sequence has indeed been shown to occur in mitochondria from peanuts and other tissues when these were suitably fortified with co-factors. Formidable problems remain in elucidating the details of utilization of the unsaturated acids, but it seems safe to conclude that the breakdown results in the production of acetyl coenzyme A (CoA)[3]. Certainly the efficiency of the overall conversion of fat to carbohydrate is such that the loss of carbon as carbon dioxide during the production of 2-C units must be very small indeed.

Now it has been established that acetate is utilized readily by slices of endosperm tissue[4]. In a 9-hr. experiment in which specifically labelled acetates were provided, it was found that at least 33 per cent of the added carboxyl carbon and 68 per cent of the added methyl carbon were converted to sucrose; the amounts converted to carbon dioxide were 46 per cent and 10 per cent, respectively. This net synthesis of sugar from acetate, with little diversion to other products, posed a problem analogous to that presented by those microbial cells which can grow on acetate as a sole source of carbon. The elucidation of the glyoxylate cycle[5], by which a net synthesis of a 4-C acid such as malate can be achieved from two acetate units, offered a basis for its solution. An examination of castor bean tissues showed that the requisite enzymes, and particularly those newly described from micro-organisms, malate synthetase and isocitratase, were indeed present. A proposal was made, therefore, that the pathway of synthesis involved the production of a dicarboxylic acid by the glyoxylate cycle followed by conversion of malate into hexose[6]. Since this time we have carried out extensive experiments on tissues of fatty seedlings and extracts which have confirmed this suggestion and firmly establish the pathway of sucrose synthesis from acetate in such material. This evidence will now be summarized.

Distribution and Capacity of the Enzyme Systems and Changes during Germination

The demonstration that isocitratase was present in the germinating castor bean raised the possibility that it was widespread in plants and accounted for the general occurrence of glyoxylate and its reduction product, glycollate. An examination of some twenty-five different tissues revealed that this was not so[7]. Far from being widespread, the enzyme was shown to be confined strictly to tissues rich in fat, particularly those of germinating seedlings. Furthermore, it was shown to be localized, in these seedlings, to those tissues in which fat is converted to sugar; it could not be detected in the adjacent parts of the seedling proper. No activity was found in the ungerminated seed or in the maturing seed on the parent plant. During the first few days of germination the enzyme activity rose dramatically and a peak was reached at about the time fat breakdown and sucrose synthesis were most rapid. Later on, as the fat content declined, so did the activity of the isocitratase. It is most significant that this decline in

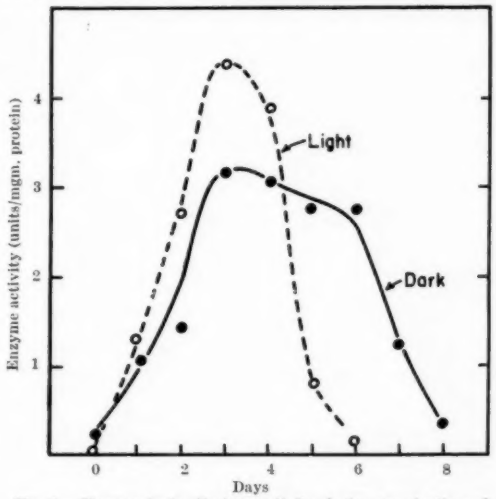


Fig. 1. Changes in isocitratase activity during germination of *Cucurbita pepo* (pumpkin) seeds
One set was exposed to light during the germination period and greening of the cotyledons occurred (ref. 7)

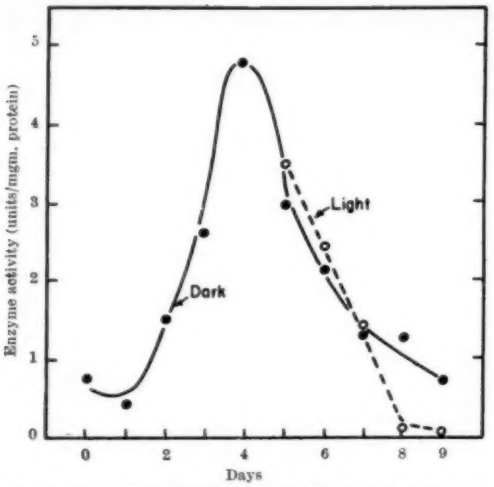


Fig. 2. Changes in malate synthetase activity during germination of *Cucurbita pepo* (water melon) seeds
One set of seedlings was exposed to light during the germination period and greening of the cotyledons occurred (Yamamoto and Beevers, unpublished)

isocitratase activity was not confined to seedlings, such as the castor bean, in which the fat reserves are in tissue which is completely absorbed during germination: it is noticed also in those seedlings the fat of which is originally present in cotyledons which persist as functioning leaves during the later growth[7]. Fig. 1 shows results obtained with seeds of the latter type. The fall in isocitratase activity occurred whether or not the cotyledons were allowed to become functional green leaves.

Malate synthetase was also found in the endosperm or cotyledon tissue of every fatty seedling examined, and it, too, showed changes in activity which paralleled the changing pace of fat breakdown[8]. It is again of interest that malate synthetase activity disappears from the cotyledons of cucurbits after the fat has been utilized (Fig. 2), since this implies a selective breakdown or inactivation of those enzymes constituting the glyoxylate by-pass at a time when other metabolic machinery is operating smoothly. Malate synthetase is not widespread in plants. Although the richest sources are fatty seedlings, it has been detected in a few tissues, for example, corn embryos, which are not primarily engaged in converting fat into sugar[8].

Isocitratase[7] and malate synthetase[9] have been partially purified from castor beans and their properties are similar to enzymes isolated from other sources. Both show an absolute requirement for divalent metal ions and are maximally activated by magnesium ions; in addition, the isocitratase shows a requirement for a compound containing —SH groups (gluthathione, cysteine). The presence of these enzymes in germinating fatty seeds has been confirmed and the amounts which can be extracted are adequate to account for the rate of sucrose production observed *in vivo*[6,8,10–12]. There is evidence that mitochondria prepared from fatty seedlings contain each of the enzymes of the glyoxylate cycle[11], but isocitratase and malate synthetase are found also in the supernatant solutions from such preparations.

Each of the enzymes which would be required to convert malate to sucrose by the following pathway has been shown to be present in the endosperm of germinating castor-oil seeds[13].

malate → oxalacetate → phosphoenolpyruvate → phosphoglycerate → di-phosphoglycerate → triose phosphate → hexose phosphate → sucrose

The formation of phosphoenolpyruvate and phosphoglycerate from oxalacetate is brought about by phosphoenolpyruvate carboxykinase. Adenosine triphosphate (ATP) is required for this step and for the formation of diphosphoglycerate in the phosphoglyceryl kinase reaction. It is of interest that two triosephosphate dehydrogenases are present, one requiring diphosphopyridine nucleotide (DPN) and the other triphosphopyridine nucleotide (TPN) as a coenzyme[14]. Of the two known systems which might bring about sucrose synthesis from hexoses, only the uridinediphosphoglucose – fructose transglucosylase was found to be present[15]. The 'assimilatory power' (ATP and reduced TPN or DPN) required for driving these reactions *in vivo* most probably is part of that produced during the oxidation of the long-chain fatty acids to acetyl CoA.

The experiments with cell-free extracts establish, therefore, that the enzymic equipment for carrying out a net synthesis of sucrose from acetate is present in the germinating castor bean. The distinctive distribution pattern and activity changes of malate synthetase and isocitratase in seedlings coincide remarkably well with the occurrence and timing of the fat → sucrose conversion. Experiments with intact tissues, which establish that such a sequence is indeed operative *in vivo*, are outlined in the next section.

Utilization of Acetate and Other Intermediates in Sucrose Synthesis

The earlier results on acetate-utilization[4] are accommodated for the sequence outlined. We have now completed an investigation of the kinetics of acetate-utilization over shorter periods in order to establish what the intermediate products are and the pathway of their interconversion. It will be realized that if the sole fate of acetate is conversion to malate in the glyoxylate cycle and then conversion to sucrose by the sequence above, the methyl carbon atom of acetate would be found only in the centre carbon atoms of malate, none would appear as carbon dioxide and it would contribute equally to carbon atoms 1, 2, 5 and 6 of the hexose units. Half the carboxyls of acetate would be lost in the conversion of oxalacetate to phosphoenolpyruvate and half would appear in carbon atoms 3 and 4 of the hexose. In the recent work with castor bean endosperm slices these predictions have been remarkably well fulfilled. In the meantime, Bradbeer and Stumpf[10] have presented valuable data from experiments with sunflower and peanuts which established that in these tissues also the products isolated after several hours in contact with labelled acetate and labelling patterns in malate and hexose were largely those predicted from the operation of the sequence as outlined.

When acetate-1-^{14}C and acetate-2-^{14}C were supplied to slices of endosperm tissue from castor bean the following results were obtained[16].

(*a*) After 2 min., and before any carbon-14 was present in sugars, virtually all the radioactivity was recovered in organic acids (other than acetate). Support for the dual entry of acetate into the gly-

oxylate cycle is provided by the finding that malate and citrate were the most heavily labelled acids.

(*b*) As shown in Fig. 3 the percentage of carbon-14 in the acid fraction declined steeply during the experiment and extrapolated clearly to 100 per cent at 'zero' time while that in the sugar fraction increased with time. This pattern establishes that a precursor–product relationship holds for the organic acids and the sugars.

(*c*) At 2 hr., when some 60 per cent of the added acetate had been utilized, the contributions of C-1 and C-2 to sucrose and carbon dioxide are those predicted (Table 1). Relatively small amounts of ^{14}C remain in other fractions; the low incorporation into amino-acids, which arise secondarily from the organic acid, should be especially noted.

Table 1. Utilization of Acetate Carbons by Endosperm Slices
(Data of Canvin and Beevers, ref. 16)

	Per cent of added carbon-14 in components after 2 hr.			
	Sugars	Carbon dioxide	Amino-acids	Organic acids
Acetate-1-^{14}C	40	2	7	10
Acetate-2-^{14}C	22	22	6	13

(*d*) Degradation of malate isolated after supplying acetate-2-^{14}C showed that at all times after 2 min. the label was equally distributed between carbon atoms 2 and 3, and even after 120 min. these carbon atoms contained more than 90 per cent of the total carbon-14 in the malate. When acetate-1-^{14}C was the substrate, the carbon-14 was exclusively confined at all times to C_1 and C_4 and equally distributed between them.

(*e*) ^{14}C-glucose-6-phosphate was detected in the acid fraction during ^{14}C-acetate utilization.

(*f*) The first labelling in the sugar fraction appeared after 5 min. exposure to the labelled acetate. At this time sucrose was labelled equally in its component hexoses but free glucose and fructose were not radioactive. At later times, when the carbon-14 in sucrose was increasing rapidly, the total radioactivity in glucose and fructose was never more than 6 per cent of that in sucrose, and the specific activity (c.p.m. per mgm. carbon) of the component hexoses was quite close to that of sucrose. Evidently, sucrose is the first and major free sugar produced, and the hexoses arise secondarily from this.

(*g*) Degradation of the glucose moiety of the sucrose produced by endosperm slices incubated with labelled acetates is shown in Table 2. At all times the distribution is close to that predicted; the methyl group of acetate is found equally in carbon atoms 1, 2, 5 and 6, while the carboxyl carbon appears equally in carbon atoms 3 and 4. (See also results from 3-hr. experiments with sunflower and peanut tissue by Bradbeer and Stumpf[10].)

Table 2. Per cent Distribution of Carbon-14 in Glucose Moiety of Sucrose Isolated from Endosperm Slices Incubated with Carbon-14 Acetate for the Times Indicated
(Data of Canvin and Beevers, ref. 16)

Substrate	Acetate-1-^{14}C				Acetate-2-^{14}C			
Carbon atom of glucose	Min. 15	30	60	120	Min. 15	30	60	120
1	3·2	5·8	4·9	8·0	22·5	21·5	21·9	22·5
2	6·0	4·8	5·3	12·2	23·8	24·5	23·8	24·0
3	32·9	37·1	38·7	35·3	2·5	4·7	6·0	3·6
4	43·5	37·6	41·6	29·2	3·7	5·3	8·8	9·6
5	7·4	8·2	5·4	7·2	23·0	21·1	20·8	16·6
6	7·0	6·4	4·0	8·0	24·5	22·7	18·8	23·4

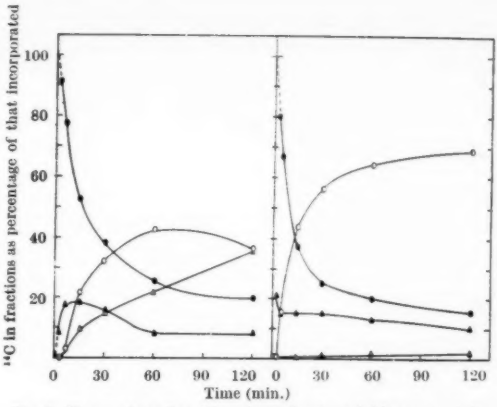


Fig. 3. Changes in carbon-14 content of water-soluble components and carbon dioxide during exposure of slices of castor bean endosperm to acetate-1-^{14}C (left) and acetate-2-^{14}C (right; ref. 16) ○, Sugars; △, carbon dioxide; ●, organic acids; ▲, amino-acids

The distinctive contributions of C-1 and C-2 acetate to the final products, the order of appearance of these carbon atoms in the various components and the degradation data are thus seen to be in good agreement with the proposed sequence.

Experiments with Other Labelled Substrates

(*a*) *Succinate and malate.* In contrast to acetate, which is rapidly absorbed and utilized, the dicarboxylic acids apparently penetrate poorly; the bulk of the added acid (about 80 per cent) was recovered from the medium, even after 8 hr. However, of the acid that did penetrate, a considerable fraction was metabolized to carbon dioxide or sugar. Furthermore, the relative contributions of the carboxyl and centre carbon atoms to these products are those predicted. Thus the carboxyl groups appeared equally in sucrose and carbon dioxide while the centre carbon atoms of succinate made a much larger contribution to sucrose[16] (Table 3).

Table 3. Distribution of Radioactivity in Carbon Dioxide and Sugar formed from ^{14}C-labelled Dicarboxylic Acids
(Data of Canvin and Beevers, ref. 16)

	Time of incubation (hr.)	Radioactivity (c.p.m.) Carbon dioxide	Sugar	Ratio Carbon dioxide/sugar
Succinate-1-^{14}C	4	617	610	1·01
Malate-^{14}C	8	1,189	886	1·34
Succinate-2-^{14}C	4	662	4,593	0·144

Table 4. Incorporation of Pyruvate Carbon Atoms into Endosperm Constituents
(Data of Neal and Beevers, ref. 17)

Labelled pyruvate	1-^{14}C	2-^{14}C	3-^{14}C
	Percentage of added radioactivity in component		
Carbon dioxide	49·3	23·8	2·9
Sugars	7·5	15·1	22·8
Organic acids	1·1	8·0	8·5
Amino-acids (alanine)	32·2	29·5	33·2

Equal samples of endosperm slices were incubated with the particular labelled pyruvate for 4 hr.

(*b*) *Pyruvate.* As shown in Table 4, pyruvate is actively converted into sucrose by endosperm slices, but the individual carbon atoms contribute in very different measure[17]. Carbon from positions 2 and 3

accounts for 85 per cent of that appearing in sucrose, with C-3 contributing about twice as much as C-2. The relative contributions to respired carbon dioxide are precisely in the reverse order. About one-third of the added pyruvate is converted to alanine (each of the pyruvate carbons contribute equally) and the acids of the glyoxylate cycle are much more heavily labelled from C-2 and C-3 of pyruvate than from C-1. It is thus seen that C-2 and C-3 of pyruvate behave in a similar manner to C-1 and C-2 of acetate. There is no evidence for a direct conversion of the intact pyruvate skeleton which would require a complete reversal of glycolysis; the conversion of C-1 of pyruvate to sucrose can be ascribed to re-fixation of the carbon dioxide released from this position[18]. The results indicate that pyruvate is converted to acetyl CoA and enters the glyoxylate cycle. Apparently the entry into the glycolytic sequence is indeed at the level of phosphoenolpyruvate, as seemed likely from other considerations[19]. On the basis of the foregoing evidence we may summarize the process of sucrose-formation from fatty acid as follows:

$$\text{C16 fatty acid} \xrightarrow{\beta\text{-oxidation}} 8 \text{ acetyl CoA} + 28\ [H]$$

$$8 \text{ acetyl CoA} \xrightarrow{\text{glyoxylate cycle}} 4 \text{ oxalacetate} + 24\ [H]$$

$$4 \text{ oxalacetate} + 8\ H \xrightarrow{\text{glycolysis reversed}} \text{sucrose} + 4\ CO_2$$

$$C_{16}H_{32}O_2 + 11\ O_2 \longrightarrow C_{12}H_{22}O_{11} + 4\ CO_2 + 5\ H_2O$$

The carbon dioxide released during fatty acid utilization is thought to arise exclusively from the oxalacetate → phosphoenolpyruvate step and thus a maximum of 75 per cent of the carbon atoms in the entering acetyl units may be converted to sucrose. Values of more than 50 per cent conversion with C-2 contributing twice as much as C-1 have in fact been observed experimentally. The gm.-for-gm. conversion of fat to sucrose is accounted for, as well as the *RQ* of 0·35 observed with endosperm slices. It should be emphasized that the quantitative agreement between the predictions of the sequence and the physiological features of the fat–carbohydrate conversion indicate that metabolism by way of the glyoxylate cycle is the fate of all of the acetate produced. The overall efficiency is such that very little must pass through the decarboxylative steps of the tricarboxylic acid cycle or be drained off in other reactions. Feeding experiments with pyruvate and acetate show that glutamate (α-ketoglutarate) production accounts for only a very small fraction of the acetate carbon utilized.

The limited distribution of the malate synthetase and isocitratase, the increasing activity and subsequent declines which they show in synchrony with the pace of fat breakdown during germination, indicate that the importance of the glyoxylate cycle in higher plants may well be confined to the part it plays during the early stages of germination of fatty seedlings.

This work was supported by grants (*G*–5072 and *G*–16149) from the National Science Foundation.

[1] Desveaux, R., and Kogane-Charles, M., *Ann. inst. nat. recherch. Agron.*, **3**, 385 (1952).
[2] Beevers, H., *Plant Physiol.*, **31**, 440 (1956).
[3] Stumpf, P. K., and Bradbeer, C., *Ann. Rev. Plant Physiol.*, **10**, 197 (1959).
[4] Beevers, H., *Biochem. J.*, **12**, 114 (1956).
[5] Kornberg, H. L., and Krebs, H. A., *Nature*, **179**, 988 (1957).
[6] Kornberg, H. L., and Beevers, H., *Biochim. Biophys. Acta*, **26**, 531 (1957).
[7] Carpenter, W. D., and Beevers, H., *Plant Physiol.*, **34**, 403 (1959).
[8] Yamamoto, Y., and Beevers, H., *Plant Physiol.*, **35**, 102 (1960).
[9] Yamamoto, Y., and Beevers, H., *Biochim. Biophys. Acta*, **48**, 20 (1961).
[10] Bradbeer, C., and Stumpf, P. K., *J. Biol. Chem.*, **234**, 498 (1959).
[11] Marcus, A., and Velasco, J., *Biochim. Biophys. Acta*, **28**, 365 (1960); *J. Biol. Chem.*, **235**, 563 (1960).
[12] Heydeman, M. T., *Nature*, **181**, 627 (1958).
[13] Benedict, C. R., and Beevers, H. (in the press).
[14] Benedict, C. R., Yamamoto, Y., and Beevers, H. (in the press). Benedict, C. R., and Beevers, H., *Fed. Proc.* (1961).
[15] Canvin, D. T., and Beevers, H., *J. Biol. Chem.*, **236**, 988 (1961).
[16] Benedict, C. R., and Beevers, H. (in preparation).
[17] Neal, G. E., and Beevers, H., *Biochem. J.*, **74**, 409 (1960).
[18] Stiller, M. L., Neal, G. E., and Beevers, H., *Plant. Physiol.*, **33**, xxiv (1958).
[19] Krebs, H. A., and Kornberg, H. L., *Energy Transformation in Living Matter* (Springer-Verlag, Berlin, 1957).

RADIOCÆSIUM AND POTASSIUM IN NORWEGIANS

By JOHAN BAARLI* and KJELL MADSHUS

Norsk Hydro's Institute for Cancer Research, Norwegian Radium Hospital, Oslo, Norway

AND

KURT LIDÉN and RICHARD C. McCALL

Radiation Physics Department, University of Lund, Sweden

As a result of nuclear weapons tests, large amounts of fission products have been introduced into the atmosphere. Up to the end of 1958 more than 250 test explosions had taken place and fission materials equivalent to 92 MT TNT had been released[1].

The fission products, once introduced into the troposphere and stratosphere, have in turn given rise to a world-wide surface deposition of isotopes. The contamination of plants and soil with these isotopes has, either directly or through selective uptake, made these isotopes available in the human diet and in the human body itself.

Among the various isotopes present in fall-out, strontium-90 and cæsium-137 are of special interest due to their long half-lives, their relatively high fission yields and similarities to important body constituents. Strontium-90 is chemically and metabolically related to calcium and must therefore be classified as a bone-seeker, while cæsium-137 behaves similarly to potassium and concentrates mainly in the muscle, liver and spleen.

The introduction of cæsium-137 into the biosphere has received considerable attention, and the mechanism of uptake of this isotope from soil through plants and animals has been discussed in detail in an effort

* Present address: CERN, Geneva, 23.

Table 1. RESULTS OF WHOLE-BODY MEASUREMENTS OF A GROUP OF NORWEGIANS FROM OSLO AND BERGEN

Subject	Sex	Date of measurement	Age (years)	Weight (kgm.)	^{137}Cs (nc.)	K (gm.)	^{137}Cs/K pc./gm.)	gm. K/kgm.	Residence	Milk (l./day)	Goat's cheese (gm./day)
K. M.	M	17/1/61	26	75·5	106·0	131	809	1·74	Bergen	1·0	40
E. F.	M	18/1/61	23	78·0	72·0	143	504	1·83	,,	1·0	7
T. S.	M	17/1/61	29	76·5	59·8	132	453	1·73	,,	1·0	7
M. R.	M	18/1/61	26	63·0	49·0	119	415	1·89	,,	0·25	0
E. L.	M	17/1/61	23	83·0	51·2	131	391	1·58	,,	0·50	0
I. L.	F	30/11/60	38	54·0	20·8	71	293	1·32	,,	0·50	0
B. M.	F	16/1/61	23	56·5	27·2	79	344	1·40	Oslo	0·30	40
B. W.	M	25/11/60	48	83·5	31·6	133	238	1·59	,,	0·30	25
U. N.	F	20/1/60	27	63·0	19·4	86	226	1·36	,,	0·20	0
T. M.	M	19/1/61	26	62·0	27·5	122	225	1·97	,,	0·75	0
M. S.	M	18/1/61	20	76·5	23·3	129	181	1·69	,,	1·0	0
G. G.	M	16/1/61	29	91·0	26·5	148	177	1·63	,,	1·0	40
J. B.	M	17/1/61	40	86·5	24·2	137	177	1·58	,,	0·30	0
K. M.	M	4/10/60	35	64·5	21·8	126	173	1·95	,,	0·30	25
I. K.	M	16/1/61	22	77·0	24·3	139	175	1·81	,,	0·75	50
S. S.	F	20/1/61	27	69·0	14·5	93	156	1·35	,,	0·50	0
K. M.	F	19/1/61	23	61·0	12·8	82	156	1·35	,,	0·75	6
K. H.	M	18/1/61	21	63·5	15·7	102	154	1·61	,,	1·6	7
O. S.	M	20/1/61	24	75·5	19·5	128	152	1·70	,,	1·0	0
S. H.	F	16/1/61	23	56·5	10·4	73	142	1·29	,,	0·50	0
T. B.	M	19/1/61	17	73·0	11·3	147	77	2·01	,,	1·0	0

to evaluate the relationship between body burdens in man and deposition of fall-out[2]. Several uncertainties seem to exist, among them the discrimination factor of cæsium-137 relative to potassium through the food chain and the rate of elimination of cæsium from the body.

The presence of cæsium-137 in various foodstuffs and also in human beings has been reported by several investigators[3–6]. Since cæsium-137 is a γ-emitter, its radiation can be detected in bulk materials by ordinary γ-spectroscopic means. Heavily shielded scintillation counters in connexion with multichannel analysers are mainly used for that purpose, and measurements of content of cæsium-137 in man are at present being made in the United States, Sweden, Britain, Germany, Canada and France.

Due to the chemical similarity between cæsium and potassium it is usual to state the concentration of cæsium-137 relative to potassium in the body. This partially eliminates effects due to differences in body-weights and simplifies comparison of body-burdens in different subjects. The results, usually expressed as pc. cæsium-137/gm. potassium to-day show a mean value of approximately 50 for persons without any occupational exposure to cæsium-137 in the countries mentioned. However, reports have been published on special cases where this number has been found to be considerably higher. In connexion with the Windscale accident, a value of 443 pc./gm. potassium was reported with a pre-accident value of 107 (ref. 7). At Hanford, an off-site visitor was found to have a cæsium-137 content of 66 nc. and a cæsium-137/potassium ratio of 370 (ref. 8). This was traced to an extremely unusual diet and milk source.

Comparison between values for the cæsium-137 content of individuals measured at different laboratories might be open to question. Agreement between different laboratories on the standardization of point sources is not always good. Recently[9] comparison of a group of so-called standard sources from several countries showed a spread of about 20 per cent although each of the sources was supposed to be standardized to ± 5 per cent or better. In comparing measurements at various times, it should be kept in mind that the cæsium-137 body-burden was increasing until some time in the middle of 1959 and has been decreasing since.

The content of cæsium-137 in human beings has of course a close relationship to the content of this isotope in the diet, which in turn partially depends on the local fall-out level. It has been established that the distribution of stratospheric fall-out depends on the geographical location, with higher concentrations in the northern hemisphere around 30°–60° N. The fall-out is also greatly influenced by the local rainfall.

Data on the cæsium-137 content of milk, which is a significant part of the diet, have indicated a larger content of cæsium-137 in milk produced in Norway[10] and Finland[11] than in milk from many other countries. This is especially true for milk from the western part of Norway. The reasons for this difference in cæsium content between eastern and western Norway are not well understood. It is probable that the amount and nature of the rainfall is at least one cause. The different grazing habits of cows in these two areas probably also contribute to the difference. Other dairy products used in the daily diet in Norway, among them cheese, have been studied, and pure goat's cheese produced in the western and northern parts of Norway in 1960 have shown values ranging up to 41 nc. cæsium-137/kgm. cheese. A blended goat's cheese is also produced in Norway which is made of 35 per cent goat's milk and 65 per cent cow's milk. This type of cheese is produced in parts of the country having lower cæsium-137-contents. Consequently the blended goat's cheese has only 15–20 per cent of the cæsium-137 content of the pure goat's cheese. In this article and in Table 1 only pure goat's cheese is considered. Table 1 lists the amount of pure goat's cheese eaten, according to the subject's own estimate. However, the difference between the pure and the blended goat's cheese is not popularly understood, and some of these estimates may include blended goat's cheese. Measurements carried out on the cæsium-137 content in other major food products have shown considerably lower values except for reindeer meat, which seems to have activities of the same order as goat's cheese.

Preliminary measurements made at the Radiation Physics Department in Lund of the cæsium-137 content in five Norwegians indicated that Norwegian inhabitants could have higher cæsium-137 body-burdens than those reported for people elsewhere. Due to the fact that no whole-body counter exists in Norway, it was decided to send another group of Norwegians to Lund to have their body radioactivity measured in the whole-body counter there. These people, mostly 20–30 years of age, were arbitrarily selected, with one group of 6 persons from Bergen (at the west coast of Norway 60° N.) and a group of 15 from Oslo (60° N.). None was occupationally exposed to cæsium-137. A few, 3 from Bergen

and 5 from Oslo, stated that they regularly used pure goat's cheese in their daily diet.

The measurements were made in an iron room with subject sitting in the 'Argonne chair' position, the detector unit was a 5-in. diameter × 4 in. thick sodium iodide (thallium) crystal and a multichannel pulse-height analyser.

The results of the measurements are shown in Table 1. This gives the measured contents of cæsium-137 in nc. and the number of gm. potassium, together with approximate daily consumption of milk (litres) and pure goat's cheese (gm.).

As can be seen from Table 1, the average cæsium-137/potassium ratio (184 pc./gm. potassium) in the people from Oslo is considerably higher than the 1960 values of ~50 pc./gm. potassium reported elsewhere. This can be compared directly with the average cæsium-137/potassium ratio of a group of people from Lund, Sweden (56° N.), of 76 pc./gm. potassium. The average value of the cæsium-137/potassium ratio of 477 pc./gm. potassium found for the Bergen group is even higher. With one exception (I. L.) the values for the Bergen group are well above those of the Oslo people. Two of the subjects, G. G. and I. K., seem to have surprisingly low cæsium-137/potassium ratios in view of their milk and goat's cheese consumption. It has been impossible to determine what type and source of goat's cheese these subjects used; but it is probable that they used the blended type.

It is generally accepted that 55–60 per cent of cæsium-137 in the human body is caused by consumption of dairy products[12,13]. It is also stated[12] that people in the United States contain a substantially constant amount of cæsium-137 due to non-dairy sources and that the remaining fraction of their cæsium-137 burden is directly proportional to their milk input. Due to this fact and because milk constitutes a significant part of children's diet, special interest has been focused on the radioactive content in this material.

Milk samples from different parts of Norway have been analysed for their content of cæsium-137 [10,14]. Beside the usual seasonal variations which are generally established, large variations are found between samples from the western and eastern parts of the country, amounting to almost a factor of 30 (Möre, on the west coast of Norway, about 900 pc./l. Oslo, about 30 pc./l. September 1960). These variations will, on a smaller scale, also show up in the population and to some extent explain the difference between the cæsium-137-level in the Bergen people and the Oslo people. The milk distribution systems for both areas are based on local central dairies; people within the same district having equal daily intake of milk should therefore be exposed to approximately equal amounts of radioactive contaminants and thus show nearly equal body burdens. As can be seen from the measured data, this condition does not hold. A man from Bergen (K. M.) and a woman from Oslo (B. M.) show body-burdens which are almost a factor of 2 higher than the mean values obtained for people in the respective districts. These two people regularly consumed rather large quantities of pure goat's cheese; it is concluded that the excess quantity of cæsium-137 in these two cases, to some extent, can be attributed to the cheese consumption.

The cæsium-137-levels in goat's cheese from different parts of the country show even larger variations than those that have been reported for cow's milk. A calculation of the cæsium-137 excess in people, due to the cheese intake, would require a complete knowledge about place and time for production of the cheese. This has been an impossible task in all cases except for one single person (B. M.), who consumed pure goat's cheese from Hallingdal only. The content of cæsium-137 in this particular cheese has been measured. The calculation of cæsium-137 excess due to cheese consumption will, however, require detailed knowledge about the discrimination factor of cæsium-137 in going from diet to man, and the biological half-time of this isotope in the human body. Studies of the discrimination factor for cæsium-137 have been reported in the literature and values ranging from 1·8 to 3·5 have been given[2]. The biological half-time, which is closely related to the discrimination factor, will show similar variation, and reports indicate values ranging from 143 to 81 days. Any of these figures might be used, yielding an excess amount of cæsium-137 due to cheese intake ranging between rather wide limits. As a compromise calculations were carried out applying a discrimination factor of 2 and a biological half-time of 100 days. The result of this calculation gives a value of 12·2 nc. cæsium-137 for B. M. due to goat's cheese consumption. This value should be compared with 27·2 nc. from Table 1. The difference 15 nc. between the mentioned figures should then be responsible for cæsium-137 intake due to other food products; this value seems to be in reasonable agreement with measured data for females not eating goat's cheese given in Table 1.

The radiation exposure for the extreme case from Bergen was calculated using the formula given by the International Commission on Radiological Protection[16] and turned out to be 0·31 mr./week. This dose is comparable to the radiation dose caused by the potassium-40, which in this case amounts to 16 mr./year or 0·3 mr./week.

It is of interest to estimate the body-burden due to a daily intake of 50 gm. of pure goat's cheese containing 41 nc. cæsium-137/kgm., which actually has been measured. This intake of cheese is considered to be quite normal in Norway. Assuming again a discrimination factor of 2 for cæsium in relation to potassium and an elimination-rate of 0·7 per cent a day (100 days biological half-life), this will in one year accumulate to 135 nc. cæsium-137. The total body irradiation caused by this amount will be of the order of 0·38 mr./week or 20 mr./year.

This investigation was carried out while one of us (R. C. McC.) was holding a Postdoctoral Fellowship of the National Cancer Institute, U.S. Public Health Service.

[1] *Hearings before the Special Subcommittee on Radiation, Congress of the United States*, **3**, 2521 (1959).
[2] Langham, W. H., and Anderson, E. C., *Health Phys.*, **2**, 30 (1959).
[3] Miller, C. E., and Marinelli, L. D., *Science*, **124**, 122 (1956).
[4] Anderson, E. C., Schuch, R. L., Fisher, W. R, and Langham, W. H., *Science*, **125**, 1272 (1957).
[5] Burch, P. R. J., *Nature*, **183**, 515 (1958).
[6] Booker, D. V., *Physics in Medicine and Biology*, **2**, 29 (1957).
[7] Rundo, J., *Nature*, **188**, 703 (1960).
[8] Roesch, W. C., McCall, R. C., and Palmer, H. E., *HW*-67045 (1960).
[9] Liden, K., and McCall, R. C. (unpublished results).
[10] Madshus, Kj., and Baarli, J., *Nature*, **186**, 527 (1960).
[11] Miettinen, J. K., Paakkola, O., Näsänen, R., Vuorinen, A., and Merten, D., *Nature*, **189**, 324 (1961).
[12] *Hearings before the Special Subcommittee on Radiation, Congress of the United States*, **2**, 1133 (1959).
[13] McNeill, K. G., and Johns, H. E., *J. Canad. Assoc. Radiol.*, **11**, 11 (March 1960).
[14] Madshus, Kj., and Baarli, J. (in preparation).
[15] McNeill, K. G., and Green, R. M., *Canad. J. Phys.*, **37**, 528 (1959).
[16] *Health Phys.*, **3**, 15 (1959).

NEWS and VIEWS

Sir Frederick Gowland Hopkins (1861–1947)

To mark the centenary of the birth (June 30, 1861) of Sir Frederick Gowland Hopkins, "the father of British biochemistry", and as a tribute to one "whose services to nutrition enriched the well-being of mankind", Messrs. Van den Berghs, Ltd., manufacturers of vitaminized margarine, have published a 26-page memoir. It has been written by Prof. Ernest Baldwin, a former colleague of Hopkins, and it is a moving account of Hopkins as a great scientist and as a most lovable man. The booklet, which contains a reproduction taken from the well-known portrait of Hopkins owned by the Royal Society, can be obtained from Messrs. Van den Berghs, Ltd., Kildare House, Dorset Rise, London, E.C.4.

Chemistry in University College, Dublin : Prof. Eva M. Philbin

Dr. Eva M. Philbin, senior lecturer in organic chemistry in University College, Dublin, has been appointed, in succession to the late Prof. J. Algar, to the chair of organic chemistry tenable in that College. Prof. Philbin graduated with first-class honours in chemistry at University College, Galway, and afterwards was awarded the degrees of Ph.D. and D.Sc. in the National University of Ireland. After a time in industry, Prof. Philbin joined the staff of University College, Dublin, in 1945, and was later appointed senior lecturer in organic chemistry. Her research interests have been mainly in the field of flavonoids, though recently she has moved on to investigations relative to the stereochemistry of pyrans. She has held visiting and research fellowships in the United States, and at Zurich, with Prof. V. Prelog.

Agriculture at Bangor : Prof. E. J. Roberts

Prof. E. J. Roberts retires this year from the chair of agriculture at Bangor, after sixteen years of distinguished service as a teacher and a prominent figure in the farming world. He comes from the Vale of Clwyd, and graduated with honours in chemistry at Bangor in 1917. After a short period in the Navy as sub-lieutenant, he proceeded to the School of Agriculture at Cambridge, for postgraduate work under the late Prof. T. B. Wood. He was appointed lecturer in agriculture at Bangor in 1922. At the outbreak of the Second World War he was seconded as agricultural executive officer for Denbighshire. In 1942 he was appointed to the chair of agriculture at Aberystwyth, returning to Bangor when that professorship fell vacant in 1945.

Roberts's research interests have covered a wide range of problems in animal husbandry; among these were lactations of various breeds of dairy cows, flushing and fat-lamb production from grassland, and the breeding of polled Friesians and Welsh Blacks. In 1936 he was seconded to the Agricultural Research Council to make a detailed survey of grass-drying, and the monograph he produced was the most comprehensive of its kind on this subject. He has served on numerous bodies concerned with agriculture, for example, the Agricultural Improvement Council, Hill Farming Committee, the Sub-committee on Agriculture of the University Grants Committee, and the Welsh Joint Education Committee. The Arton Wilson Report (1956) on the Provincial and Local Organization and Procedures of the Ministry of Agriculture, Fisheries and Food also bears his name among its signatures. At Bangor, during the past ten years, he has carried out, by the aid of moneys from the University Grants Committee, an extensive scheme of remodelling and adaptation of the College Farm to form an efficient teaching unit.

Prof. I. A. McK. Lucas

Mr. Ian A. McK. Lucas succeeds Prof. Roberts at the comparatively early age of thirty-four. A graduate in agriculture of the University of Reading, he was a prizeman in agriculture. During 1947–49 he held a Ministry of Agriculture postgraduate scholarship in the Nutrition Department of Macdonald College of McGill University. There he took his M.Sc. under Prof. E. W. Crampton. After a short period as assistant lecturer in agricultural chemistry at Harper Adams Agricultural College, Prof. Lucas in 1950 joined the Applied Nutrition Department of the Rowett Research Institute as head of its section on pig nutrition, which post he now holds with distinction and with the rank of principal scientific officer. For a year (1957–58) Lucas, on study leave, held a New Zealand Department of Scientific and Industrial Research Fellowship at Ruakura Animal Research Station, where he broadened his experience by conducting experiments on grazing cattle and sheep. For six weeks thereafter he toured Australia at the invitation of the Australian Meat Board. He is the author of some thirty-two papers on various aspects of animal nutrition, mainly those concerned with the physiology of nutrition of the pig, particularly during early growth. Lucas is at present technical secretary of the Agricultural Research Council's Technical Committee on Nutrient Requirements of Farm Livestock and of its Working Party on Terminology. He is also a member of the Working Party on Pigs. Lucas also serves as a member of the Pig Commission of the European Society of Animal Production.

Presidency of the Mellon Institute: Dr. P. C. Cross

Dr. Paul C. Cross, of the University of Washington (Seattle), has been appointed president and chief executive officer of the Mellon Institute. Dr. Cross, a native of Bruin (Butler County), Pa., has been professor and executive officer of the Department of Chemistry of the University of Washington since 1949. He will assume his new post on September 1. He was born on July 19, 1907, received his bachelor's degree from Geneva College in 1928, and his doctor's degree from the University of Wisconsin in 1932. Afterwards, he held research fellowships at Wisconsin, the California Institute of Technology, and Stanford University. He became an assistant professor at Stanford in 1926, and in 1938 joined Brown University as an associate professor. During 1942–49 he served successively as professor, director of the Metcalf Research Laboratory, and chairman of the Chemistry Department at Brown University.

XUM

Department of Technical Co-operation

THE Department of Technical Co-operation came into being on July 24. The Minister in charge is Mr. Dennis Vosper, who will be known as the Secretary for Technical Co-operation. The director-general—the permanent head of the Department—is Sir Andrew Cohen. The Department's headquarters are at Carlton House Terrace, London, S.W.1. The new Department is taking over present work on technical assistance performed by the three overseas departments (the Foreign Office, the Commonwealth Relations Office and the Colonial Office) and the Ministry of Labour, together with their present staffs engaged on this work. The object is to enable the United Kingdom to meet more effectively requests for technical assistance received from the Governments of developing countries inside and outside the Commonwealth. Among the main activities of the Department of Technical Co-operation will be the following: (i) Recruitment of staff for service with overseas Governments and international organizations. (ii) Arrangements for the training of such staff, including staff already in the service of overseas Governments. (iii) Administration of the Overseas Service Aid Scheme which was set out in the White Paper, *Service with Overseas Governments* (Cmnd. 1193). (iv) Responsibility for the work of the Directorate of Overseas (Geodetic and Topographic) Surveys and the Directorate of Overseas Geological Surveys. (v) Aspects of Commonwealth educational co-operation affecting the United Kingdom. (vi) Special technical assistance projects overseas undertaken at the request of the Governments concerned. (vii) The handling of requests from United Kingdom dependent territories for technical assistance (including the financing of such assistance) under the Colonial Development and Welfare Acts, and from Commonwealth and foreign countries and international agencies. (viii) Making arrangements for assistance in the field of research, principally on subjects of importance to United Kingdom dependent territories, or where the United Kingdom has a special contribution to make overseas. (ix) Supplementing the work of other organizations in assistance to overseas Governments in placing students in the United Kingdom. (x) Providing assistance in certain instances to overseas Governments on technical matters relating to air and surface transport, telecommunications and postal services. (xi) Provision to overseas Governments of specialist advice at their request on a wide variety of subjects, such as agriculture, education, fisheries and medicine. Apart from certain educational projects, the new Department will not be responsible for the provision of capital aid, which will remain the responsibility of the overseas Departments.

Research and Development in Scotland

THE Parliamentary Secretary for Science, Mr. D. Freeth, replying to questions in the House of Commons on July 11, said that of 77 applications for grants for special researches received in 1960 from Scotland by the four Research Councils, 61 were accepted. Receipts from Scottish industry during 1960–61 amounted to about 4 per cent of the total receipts from industry by the Department of Scientific and Industrial Research in respect of work done at its establishments. Of 105 postgraduate awards for training in science and technology tenable at universities and colleges in Scotland made by the Department in 1960, 17 were in biology and biochemistry, 6 in chemical engineering and metallurgy, 40 in chemistry, 5 each in electrical engineering and engineering, 3 in geology, 6 in mathematics, 22 in physics and 1 in the human sciences.

Effects of Insecticides on Birds

THE Minister of Agriculture, Fisheries and Food, Mr. C. Soames, stated in reply to a question in the House of Commons on July 3 that the report of the survey of the effects of insecticides on animal life in Britain had been discussed at a meeting on June 29 with all the organizations interested. The report showed that the widespread occurrence of deaths of birds was confined to springtime, and that the main trouble could be obviated by not using dressings containing dieldrin, aldrin and heptachlor for spring sowings. Accordingly, it had been agreed that, as from January 1 next, these dressings will not be used at all for spring-sown grain, and they will only be used for dressing autumn and winter wheat where there is real danger of attack from wheat bulb fly. These arrangements will operate under the terms of the Notification of Pesticides Scheme. It has also been agreed that the Ministry should continue its survey, and that the arrangements should be reviewed at a meeting in June 1962. Pressed on various points, Mr. Soames promised to consider carefully the recommendations of the Estimates Committee regarding the voluntary schemes, but his information was that they had worked.

Farm Buildings in Britain

IN reply to a question in the House of Lords on July 6, the Minister for Science, Lord Hailsham, said that the Government does not think that a case has been established for a Government farm buildings research centre, but the Agricultural Research Council is undertaking a survey of farm buildings in Great Britain and is issuing a bibliography of research publications on the subject. The Council, in co-operation with the farmers concerned, is also organizing experiments with buildings on farms and contributing towards their capital cost. Lord Hailsham said further that with the experimental scheme started last year, by which landowners interested in scientific experiment could receive a 50 per cent grant for genuinely experimental buildings, the owners had to work in co-operation with the Agricultural Research Council. This method, covering the whole of Britain, with its different types of farming, circumstances and climate, appears to offer much better value than a research centre and the scheme has given very promising results. Of 177 applications received, 27 had been accepted as suitable for grants, 8 withdrawn and 90 rejected; 52 are under consideration.

Bibliography of Farm Buildings Research 1945–58

THE Agricultural Research Council is compiling a general bibliography of research publications on farm buildings. This is the first bibliography of the subject to be compiled, and *Buildings for the Drying and Storage of Grain* is the fifth section of the bibliography to appear. It will be followed by a section dealing with buildings for the processing and storage of fodder and a general section which will deal with miscellaneous types of building and with the construction and maintenance of farm buildings. The bibliography regards farm buildings as part of farm equipment

and gives references to any matter affecting their design, construction, economics and use. It includes the findings of scientific research, of such systematic but less-precise forms of investigation as surveys, and of field trials and similar developmental phases of new techniques. It includes information from any country where conditions are relevant to Great Britain. Abstracts of the original material and a review summarizing the main conclusions are provided. The present bibliography covers the period January 1, 1945, to April 30, 1958. The Council intends to keep it up to date by publishing supplementary bibliographies from time to time. *Buildings for the Drying and Storage of Grain* (3*s*., postage 3*d*.) is obtainable from the Agricultural Research Council, Cunard Building, 15 Regent Street, London, S.W.1.

Public Health as a World Problem

THE fourth report from the World Health Organization's Expert Committee on Public Health Administration, of which Sir John Charles is chairman, is entitled *The Planning of Public Health Services* (Technical Report Series, No. 215. Pp. 48. Geneva: World Health Organization; London: H.M.S.O., 1961. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars). It reviews broadly the experience in this field of Ceylon, India, Czechoslovakia, the U.S.S.R., the United Arab Republic and the United Kingdom, before considering in detail the general principles of planning such services in countries at varying stages of development and with different social and economic systems, to which any planning must be related. Stress is laid on the use of surveys to secure the information on which planning must be based, the importance of securing full co-ordination and collaboration from the medical and other professions concerned and the interest and understanding of the general public. The determination of priorities and the choice of long-term targets are also necessary, and since planning is a continuous process arrangements should be made for continuous review and assessment, so that the health programme is kept constantly in line with changing conditions; and in this connexion the value of operational research is emphasized. It is no longer satisfactory to plan health services on an empirical basis, and further research in public health service is needed to provide indicators for assessing on a more scientific basis the level of public health, the efficiency of health services, standards, and the economics of disease and disability and their reduction.

National Foundation for Scientific Research, Belgium

THE thirty-third annual report of the Belgian National Foundation for Scientific Research, covering the year 1959–60, comprises chiefly alphabetical lists of recipients of grants and of their publications during the year, in which grants totalling 39 million francs were voted (Fonds National de la Recherche Scientifique. Trente-troisième Rapport Annuel, 1959–60. Pp. 284. Bruxelles: Fonds National de la Recherche Scientifique, 1961). Of this total, 382,500 francs was to the Unive sity of Ghent, 240,000 francs to the University of Liège, 730,000 francs to the University of Brussels and 850,000 francs to the University of Louvain. Special grants noted in the report include 1,000,000 francs to the Committee for the Study and Exploitation of Electronic Calculators; 1,875,000 francs for research programmes concerned with the technique of regulation (chemical processes and stochastic phenomena) of the transmission of heat, automobile suspension and the mechanics of flight; 300,000 francs for studies of the effect of *P*, *Q*, *R*, *S*, *ST* and *T* waves of the vectorial electrocardiogram of man, the dog and the rabbit; 2,175,000 francs for a study of dynamic and static deformations, stresses, vibrations and temperatures in construction; 500,000 francs for studies to determine the structure of natural products (sesquiterpenes, steroids, triterpones and alkaloids); and 300,000 francs for a study of the diffusion of atoms and free radicals in the solid phase at 4° K. as a function of their masses. There is also a note on research during the year at the Jungfraujoch scientific station.

Teachers and Health Education

HEALTH education is not always recognized as a discipline of its own and given its appropriate place in the training of teachers. A new report (Joint WHO/Unesco Expert Committee on Teacher Preparation for Health Education. World Health Org. Tech. Rep. Ser., 1960. No. 193. Pp. 19. 1*s*. 9*d*. Published also in French and Spanish) provides a flexible framework of guiding principles for such training, which should take place not only through courses of instruction but also through the teacher's actual mode of life in his training institution, his contact with student health services, and his experiences when practising teaching. Health education in schools should be based on the health needs and interests of the children, the teacher having many opportunities for imparting it both directly and by the part he can play in the school health services and in planning for a healthy school. To enable him to make the most of his opportunities, he must have some knowledge of growth and development, personal and community health, school health practice, and methods of health education. The report discusses these subjects, the future teacher's life as a student in relation to his opportunities for health work and to student health services, practice teaching, and the education he acquires as a fully-fledged teacher. Other sections deal with planning of training for teachers in health education, the desirability of one person in each training institution being responsible for co-ordination of courses and activities in health education, and possible future national and international developments, studies and research in the subject.

Hospital Efficiency Studies

THE Ministry of Health has issued recently, to hospital authorities, a second set of *Abstracts of Efficiency in the Hospital Service*. The first set, comprising seven investigations, was published in April. The aim of the series is to enable all hospitals to profit from simplification, improvements and money-saving ideas which have been found practicable in the hospital field and to reduce the risk of related measurements and tests being unnecessarily repeated. All the hospital authorities have been asked to contribute to the series by supplying brief factual details from studies they have undertaken. The second set is made up of six studies. These cover: maintenance of grounds and gardens; document copying; medical stenographers' department; medical records (including mobile steel-shelving and addressing machine systems); and, finally, the collection of swill from hospital wards.

XUM

How Old are the Angiosperms?

In the first of two recently published papers a fossil nut which looks very like some of the modern Icacinaceae is described ("An Early Cretaceous (Hanterivian) Angiosperm Fruit from California", by M. E. J. Chandler and D. I. Axelrod; "How Old are the Angiosperms?" by D. I. Axelrod. *Amer. J. Sci.*, 259, 441; 1961). This tropical family is regarded as moderately advanced, and if the fossil (which is classified with great caution) is rightly placed it fits very nicely with the thesis of the second paper. In this, Axelrod defends his well-known idea that the flowering plants arose in the 'uplands' during the late Palæozoic (and being upland were very seldom preserved). Here in the uplands they evolved most of their families during the early Mesozoic, and when they appear in force in the Upper Cretaceous this is due to their conquest of the delta habitat rather than to evolution.

Axelrod's arguments had recently been attacked by Scott, Barghoorn and Leopold ("How Old are the Angiosperms?", *Amer. J. Sci.*, 258, A, 284; 1960), and a good deal of the present paper can be crudely summarized thus: lack of evidence can be explained away (and in several different ways); and if certain early fossils supposed to be Angiosperms have not been strictly proved to be Angiosperms, neither have they been strictly proved not to be Angiosperms. To the reviewer it looks as if at least as much thesis has by now been built on the early fossils as they will support, and what is needed is more support; not perhaps by more early fossils but by fuller knowledge of those we have. If, for example, the fossil nut in the first paper was shown by further knowledge to agree with the Icacinaceae and with them exclusively, we would have a very useful basal fact.

Yorkshire Philosophical Society

The report of the Council of the Yorkshire Philosophical Society for 1960 rightly states that it was an important year for the Society. By a mandate given at a special general meeting in December 1959, an agreement was made with the Corporation of York for it to take over the future maintenance of the Yorkshire Museum, its collections and Gardens. They will be managed by a joint committee, one-third of the members of which will be nominated by the Council of the Society. Founded in 1822, the decision to give the Museum and Gardens to the City after 139 years of an existence entirely supported by members is a historic one and the occasion was marked by appropriate ceremony. It is felt that the community at large will benefit greatly by such a gift, and the Society will be free to develop its separate interests. An unexpected discovery in the excavations in the Garden was an apparently late-fourth-century ditch. It is hoped that further work will shed more light on this last phase of Roman York.

International Business Machines, United Kingdom, Ltd.

International Business Machines, United Kingdom, Ltd., have announced the foundation of three research fellowships, one in each of the years 1961, 1962 and 1963, tenable at any university or university college in the United Kingdom, under a continuing scheme (*IBM Research Fellowships*. Pp. 4. London: IBM United Kingdom, Ltd., 1961). The fellowships, which will carry a salary of not less than £1,000 a year, will be in no way restricted as to subject, and will be of sufficient duration to allow research into major problems to be undertaken. The 1961 fellowship has been awarded to the Department of English and General Linguistics at the University of Edinburgh. The facilities of the full range of installed International Business Machine equipment are also to be made available free of charge for approved academic and humanitarian research of a non-profit-making nature. The terms of this Endowed Research Time scheme are set out in a further booklet (*Endowed Research Time*. Pp. 3. London: IBM United Kingdom, Ltd., 1961).

Research Grants in Radiological Health

In view of the rapid expansion which is forecast in the use of nuclear energy, X-rays and other sources of radiation, a greatly expanded programme for research grants in the field of radiological health has been developed by the U.S. Public Health Service. These grants are offered to support research by individuals, universities, hospitals, laboratories and other public or private institutions in the assessment and control of the effects of man-made and natural radiation on the individual. Research proposals should contribute to the determination of the extent and character of the radiation problem as well as the mechanisms by which radiation produces damage. Information and application forms can be obtained from Dr. Paul F. Hahn, chief of the Office of Extramural Grants, Division of Radiological Health, U.S. Public Health Service, Washington 25, D.C.

American Grants for Nuclear Equipment in Argentina, Brazil and Israel

The United States is to make available to the International Atomic Energy Agency approximately 80,000 dollars-worth of nuclear equipment to assist in developing the peaceful uses of atomic energy in Argentina, Brazil and Israel. Argentina will receive a grant for a mass spectrometer ion-source to be used in the electromagnetic separation of short-lived radioactive material, and an electron paramagnetic resonance spectrometer to be used in connexion with solid-state physics work being conducted at the Institute of Physics of San Carlos de Bariloche. The grant to Brazil will provide equipment for research in the use of isotopes in agriculture. Israel will receive equipment for one mobile and two stationary monitoring stations as part of its radiological protection programme to provide routine monitoring of the area surrounding the swimming-pool-type research reactor located near Rehovoth, Israel.

Monograph Prizes

The American Academy of Arts and Sciences is offering three 1,000-dollar prizes, to be awarded annually to the authors of unpublished monographs —one each in the fields of the (1) humanities, (2) social sciences, (3) physical and biological sciences. The final date in 1961 for receipt of manuscripts by the committee on awards is October 2. Details can be obtained from the Committee on Monograph Prizes, American Academy of Arts and Sciences, Little Hall 33, Harvard University, Cambridge 38, Mass.

NATO Advanced Study Institute Programme, 1962

Short courses at which scientific topics are discussed at an advanced level may be eligible for

financial support under the NATO Advanced Study Institute Programme. Courses should last for two weeks or longer, and participants should be drawn from several countries. The NATO grant may be used for administrative expenses, publication of the proceedings, and to subsidize the expenses of foreign visitors. Courses in any of the sciences, for example, mathematics, astronomy, physics, chemistry, biology, etc., are eligible for support. The meetings may consist of experimental classes, as well as the more usual arrangement of lectures and discussions. The Programme, which this year is supporting nineteen courses, will continue in 1962, subject to confirmation from the appropriate authorities. It is hoped to raise the budget-level of the Programme. Persons interested in organizing such courses and requiring financial assistance from NATO should write, before December 1, to the Scientific Affairs Division, NATO, Place du Maréchal de Lattre de Tassigny, Paris 16e, France.

Royal Meteorological Society: Officers for 1961–62

THE following have been elected officers and Council for 1961–62 of the Royal Meteorological Society: *President*, Dr. H. L. Penman; *Vice-Presidents*, Dr. A. G. Forsdyke, P. J. Meade, J. S. Sawyer, Dr. J. M. Stagg; *Vice-President for Canada*, Dr. F. K. Hare; *Vice-President for Scotland*, R. Cranna; *Treasurer*, B. C. V. Oddie; *Secretaries*, Dr. R. C. Frith, Dr. R. S. Scorer, Dr. F. Pasquill (*Editor*), G. A. Bull (*Librarian*); *Ordinary Members of Council*, Dr. J. A. Chalmers, T. J. Chandler, R. H. Clements, J. R. D. Francis, W. A. Grinsted, Dr. J. T. Houghton, E. Knighting, A. L. Maidens, Dr. J. L. Monteith, P. R. Owen, Inst. Capt. R. Thorp, Dr. G. B. Tucker.

University News :

Edinburgh

THE following have been made readers within their respective faculties, with effect from October 1: (*a*) Faculty of Medicine, Dr. Eric Samuel, honorary senior lecturer in radiodiagnosis (status of honorary reader), Dr. H. M. Adam, senior lecturer in pharmacology, and Dr. J. S. Robson, senior lecturer in therapeutics; (*b*) Faculty of Science, Dr. N. A. Mitchison, lecturer in zoology.

Appointments have been made as follow: Dr. P. P. Brown, lecturer in bacteriology; Dr. W. P. Doyle and Dr. J. L. Holmes, lecturers in chemistry.

The Department of Radiotherapy has received an anonymous gift of £1,000 for research.

Royal College of Science and Technology, Glasgow

DR. DAVID W. A. SHARP has been appointed senior lecturer, and Dr. Graham R. Knox lecturer, in the Department of Chemistry from September 1.

London

THE following have been appointed readers in the Imperial College of Science and Technology : Dr. C. B. Alcock (chemical metallurgy), Dr. J. N. Hunt (physical oceanography), Dr. J. A. Kitchener (science of mineral processing).

Manchester

MR. R. H. KANTOROWICH, formerly lecturer in town planning in the Department of Land Surveying and Civil Engineering at the University of Cape Town, has been appointed professor of town and country planning in succession to the late Prof. Clifford Holliday.

Southampton

THE following appointments have been made: Mr. B. M. Bland, at present deputy librarian in the University of Birmingham, to the University librarianship; Mr. H. J. Trump, at present director of extra-mural studies in the University of Glasgow, to the directorship of adult education; Dr. J. P. Jones, senior lecturer in the Department of Aeronautics and Astronautics, to the Westland readership in helicopter engineering. The title of reader has been conferred on Dr. I. G. M. Campbell, senior lecturer in the Department of Chemistry.

Announcements

SIR RUDOLPH PETERS, emeritus professor of biochemistry in the University of Oxford, and Dr. J. L. Pawsey, assistant chief of the Division of Radiophysics, Commonwealth Scientific and Industrial Research Organization, Sydney, have been awarded the degree of Hon. D.Sc. of the Australian National University at Canberra.

MR. DAVID OWEN, assistant head of the Department of Operational Research and Cybernetics of the United Steel Companies, Ltd., has been appointed head of the Department in succession to Mr. Stafford Beer, who is to be managing director of a new international firm of operational research consultants based in London.

DR. B. J. A. BARD, vice-chairman of Dracone Developments, Ltd., has been appointed chairman of the company, in succession to Prof. W. R. Hawthorne, professor of applied thermodynamics in the University of Cambridge, the inventor of the Dracone project, who will continue on the board of the company as technical adviser.

MR. N. G. MCCULLOCH has been appointed an honorary life governor of the Cotton, Silk and Man-made Fibres Research Association, Shirley Institute, Manchester, in recognition of his outstanding services to the Association and the industry. Mr. McCulloch has been intimately associated with the Shirley Institute for more than twenty years and played a leading part in the discussions which led to the recent fusion of the British Cotton Industry Research Association and the British Rayon Research Association.

THE Faraday Society has arranged a general discussion on "The Structure and Properties of Ionic Melts", to be held in the Department of Inorganic and Physical Chemistry, University of Liverpool, during September 5–7. Further information can be obtained from the Secretary, Faraday Society, 6 Gray's Inn Square, London, W.C.1.

AN international conference on plasma physics and controlled nuclear fusion research will be held by the International Atomic Energy Agency at Salzburg, Austria, during September 4–9. It is expected that the conference will show what progress has been achieved since the Second United Nations International Conference on the Peaceful Uses of Atomic Energy, held in Geneva in the 1958. The proceedings of the conference will appear in special issues of *Nuclear Fusion : Journal of Plasma Physics and Thermonuclear Fusion*, published by the Agency. Further information can be obtained from the Division of Public Information, I.A.E.A., Vienna I, Kärntnorring 11, until August 31, and afterwards at Europahaus, Salzburg, Austria.

XUM

WATER RESOURCES OF THE UNITED STATES

As a result of Senate Resolution No. 48 of the Eighty-sixth Congress of the United States, a Select Committee on National Water Resources was set up in 1959 to study the extent and character of water resources activities required to provide the future quantity and quality of water needed for all segments of the economy in the United States.

Under the vigorous leadership of Senator Robert S. Kerr from Oklahoma, this Committee has now completed its assignment and presented its final report and recommendations to the first session of the Eighty-seventh Congress. The final report was preceded by a series of specialist reports which have been appearing steadily over the past two years. A number of these reports has already been reviewed in *Nature* (**187**, 562; 1960), and have been marked with an asterisk in the now complete list given here. Many of these reports and also the final recommendations will be of interest to those faced with water problems in the United Kingdom and Commonwealth as well as elsewhere. A brief outline of the salient points of the new reports and the final recommendations therefore follows.

List of Committee Prints issued by the Select Committee on National Water Resources, U.S. Senate, 1959–60, under the title *Water Resources Activities in the United States*. Obtainable from the Government Printing Office, Washington

(*A*) Background

1. *Water Facts and Problems*, U.S. Geological Survey.
* 2. *Reviews of National Water Resources during the Past Fifty Years*. Legislature Reference Service, Library of Congress.
* 3. *National Water Resources and Problems*, U.S. Geological Survey.
* 4. *Surface Water Resources of the United States*, U.S. Geological Survey.
* 5. *Population Projections and Economic Assumptions*, Census Bureau and Resources for the Future.
6. *Views and Comments of the States*, State reports on their water resources and problems.

(*B*) Projections of Future Needs

* 7. *Future Water Requirements for Municipal Use*, Public Health Service.
8. *Future Water Requirements of Principal Water-Using Industries*, Business and Defence Services Administration and Bureau of Mines.
* 9. *Pollution Abatement*, Public Health Service.
10. *Electric Power in Relation to the Nation's Water Resources*, Federal Power Commission and others.
11. *Future Needs for Navigation*, Corps of Engineers, U.S. Army.
* 12. *Land and Water Potentials and Future Requirements for Water*, Department of Agriculture.
* 13. *Estimated Water Requirements for Agricultural Purposes and their Effects on Water Supplies*, Department of Agriculture.
14. *Future Needs for Reclamation in the Western States*, Bureau of Reclamation.
15. *Floods and Flood Control*, Corps of Engineers, U.S. Army.
* 16. *Flood Problems and Management in the Tennessee River Basin*, Tennessee Valley Authority.
17. *Water Recreation Needs in the United States*, National Park Service.
18. *Fish and Wildlife and Water Resources*, Fish and Wildlife Service.
* 19. *Water Resources of Alaska*, U.S. Department of the Interior.
* 20. *Water Resources of Hawaii*, U.S. Department of the Interior.

(*C*) Techniques for Meeting Needs

* 21. *Evapo-Transpiration Reduction*, Department of the Interior and Department of Agriculture.
* 22. *Weather Modification*. Weather Bureau and Dean A. M. Eberle.
* 23. *Evaporation Reduction and Seepage Control*, Bureau of Reclamation.
* 24. *Water Quality Management*, Public Health Service.
* 25. *River Forecasting and Hydrometeorological Analysis*, Weather Bureau.
* 26. *Saline Water Conversion*, Office of Saline Water, Department of the Interior.
* 27. *Application and Effects of Nuclear Energy*, Atomic Energy Commission.
* 28. *Water Resources Research Needs*, Department of Agriculture.
29. *Water Requirements for Pollution Abatement*, Prof. George Reid, University of Oklahoma.
30. *Present and Prospective Means for Improved Re-use of Water*, Abel Wolman Associates.
31. *The Impact of New Techniques on Integrated Multiple-Purpose Water Development*, E. A. Ackerman and Associates.
32. *The Supply and Demand of Water in the United States*, Dr. Nathaniel Wollman, Resources for the Future.

(*D*) Hearings of Evidence

Hearings Held by the Select Committee on National Water Resources and published in 23 parts with index. Pp. 3968. (Washington, 1960.)

(*E*) Final Report and Recommendations

Report of the Select Committee on National Water Resources, Report No. 29 of the First Session of the Eighty-seventh Congress, 1961.

Committee Print No. 1 consists of copies of the charts prepared by the U.S. Geological Survey on the occasion of the presentation of an account of the water resources and related problems of the United States at a meeting in July 1959. Much of the material in this report is also covered in Print No. 3, which has already been reviewed.

Committee Print No. 5 contains the population projections and economic assumptions on which the studies for the water resources committee are based. This report is of considerable interest and has wider implications beyond that of the water problem. The first part predicts that the population of the United States will reach between 225 and 278 million by 1980, and between 267 and 431 million by the year 2000. Breakdowns of these projections by States and water resource regions, and between urban and rural areas in each water resource region are given. The report covers projections made by the U.S. Census Bureau, the Committee Staff and 'Resources for the Future', a private organization.

Committee Print No. 6 is a voluminous report detailing the views and comments of each State on the water problem. The replies received from the invitation to submit evidence range from brief letters to elaborate treatises from States where water is an acute problem. Taken as a whole the responses provide a valuable background to the national problem.

Report Nos. 8, 10 and 11 form a natural group dealing with industry, electricity and navigation, respectively. Committee Print No. 8 attempts an assessment of the future water requirements of the principal water-using industries. The 1954 census showed a total use of water of more than $12{,}000 \times 10^9$ gal. annually in the primary manufacturing industries, exclusive of electric power generation. The Department of Commerce estimates that the 1954 intake of $7{,}700 \times 10^9$ gal. for the iron and steel, chemical, pulp and paper, food and beverage, aluminium and copper industries could rise to $26{,}500 \times 10^9$ gal. by 1980 and $61{,}600 \times 10^9$ gal. by the year 2000. The Bureau of Mines projects water intake in the mining industries from $1{,}790 \times 10^9$ gal. in 1954 to $3{,}765 \times 10^9$ gal. in 1980 and $4{,}770 \times 10^9$ gal. in the year 2000. Taking all industries exclusive of electric power together, a total need of water of up to $36{,}500 \times 10^9$ gal. a year by 1980 and double this amount by the year 2000 is estimated. Committee Print No. 10 deals with the needs of the electric power industry, and it is not surprising to see even more astronomical figures. During 1959 about $26{,}813 \times 10^9$

gal. of water were circulated in thermal plants of investor-owned systems. By 1980 these will need $106{,}409 \times 10^9$ gal. yearly. To this must be added the needs of nuclear power plants and the rural electrification schemes of the Department of Agriculture. Fortunately, from the point of view of national water supplies, most of this water is needed for cooling purposes, and less than 1 per cent is a consumptive use. The same consideration also applies to hydro-electric developments: the report shows that present hydropower capacity represents between one-quarter and one-third of the actual and economic water-power potential, and that the overall resource limitation on hydro development in the United States is not yet within sight. Committee Print No. 11 details the future needs for inland navigation. This report covers briefly the history of navigation on the inland waterways system of the United States (which extends to some 20,000 miles), gives an inventory of the present system, and discusses possible improvements and extensions thereto in the light of the estimates that freight traffic will double by 1980 and quadruple by the year 2000. The improvement of some 10,041 miles of existing waterways and the creation of some 3,070 new waterways might eventually be needed.

Committee Print No. 14 investigates the future water needs for reclamation in seventeen Western States. These States have had in recent years a more rapid growth in population and economic activity than the United States as a whole, and the trend is likely to continue. The Bureau of Reclamation, which has been responsible for much of this development, was asked, therefore, to report on anticipated future developments of reclamation in the Western States between now and the year 2000, including Federal, State and local projects. In preparing its report, the Bureau has inventoried more than 1,000 projects, of which it estimates that some 800 will be realized by the year 2000, bringing in 17 million acres of newly irrigated land and creating 230,000 family-sized farms for an 'on-farm' population of 800,000. This could support a local non-farm population of 2·4 million and provide food and fibre for 25 million people. The full efficient utilization of water resources in the Western States would require significant changes in existing patterns of use of water. Many involved water exchanges would have to be worked out, the State laws with respect to ground water would need modification, a good deal of research on the hydrological cycle would be necessary and many financial problems would need resolution. Appendixes to the report include maps and charts showing the location and construction costs of the 1,085 projects. This report has proved to be one of the most interesting, stimulating and also challenging accounts of the whole series.

Committee Print No. 15 discusses the flood problems of the United States, tabulates past and expected future flood damage by river basins, presents the historical and legislative background of flood control in the United States, and considers possible solutions for flood problems in the light of the fact that overall flood damage is increasing more rapidly than it has been possible to reduce it by the construction of control works. Three alternative methods of reducing damage exist: (*a*) abandonment or evacuation of flood plains; (*b*) regulation of flood plain use to accommodate the use to the flood hazard; (*c*) provision of works and measures for flood protection or control. The report presents an inventory of works and projects needed to prevent flood damage in the foreseeable future until 1980. These works include more than 64 million acre-feet of flood control storage in reservoirs, 4,300 miles of levees and flood walls, and 5,900 miles of channel improvements.

Committee Prints Nos. 17 and 18 serve as salutary reminders that the water problem is not confined to industrial and agricultural interests. The rapidly rising demand for out-door recreation by an expanding population with a rising standard of living is considered in Report No. 17. There are few outdoor activities from which water is absent, and the report sets forth recommendations from the National Park Service regarding future water recreation needs. It suggests that some 15 per cent of the oceanic and major inland water shoreline should be acquired, that certain free-flowing streams the recreational value of which outweighs other considerations should be preserved, that water reservoirs should where possible be made multi-purpose, that efforts to combat pollution should be increased. Little consumptive water is involved in the report, which is concerned mainly with the need for conservation of water for recreation. Committee Print No. 18 deals with a rather more specialized aspect of outdoor recreation—that of fish and wild life in relation to water resources. The report points out that the problem of maintaining fish and wild life resources are compounded by the combination of the rapidly increasing population and increase in leisure time together with the gradual and increasing encroachment of man's activities on the habitat available for fish and wild life. It is urged that fish and wild-fowl conservation and development should be considered as a co-equal purpose with other demands on water resources development in the United States.

Committee Print No. 29 on pollution abatement supplements Nos. 9 and 24 previously reviewed. One of the principal requirements for water in the future is for the dilution of effluent resulting from the treatment of municipal sewage and industrial wastes which are disposed of into rivers. Prof. Reid, who with the advice of technical experts from the Public Health Service, developed short-cut methods for estimating dilution water requirements, is largely responsible for report No. 29. The study is limited to the rivers in the 22 water resource regions delineated and excludes the problems of tidal estuaries. Waste loadings that would normally be discharged directly into the sea or large lakes were not considered.

Committee Print No. 30 is an important account of present and prospective means for the re-use of water. As demands for water rise, it becomes increasingly necessary for the public to use second-hand water. With all the indications pointing towards a three-fold increase in water withdrawal by 1980, rising to a six-fold increase by the year 2000, the need for improving techniques of water re-use is obviously urgent. The report indicates that already 70 million of the 117 million people now served by public water systems are re-using water that may have passed through a domestic sewage or industrial waste disposal plant at least once. The report is based on a review of several thousand technical papers: it presents methods of reclaiming water from sewage and industrial wastes, outlines the problems encountered in the re-use of water, goes into considerable detail on the restoration and maintenance of underground water

supplies, covers ground water recharge, and reclamation of saline waters.

As a fitting companion to Committee Print No. 30 we find No. 31 dealing with the impact of new techniques on integrated multiple-purpose water development. To meet a situation within this century in which water requirements will cause the United States to go beyond the traditional approaches to water development, the report suggests a new type of approach termed "comprehensive development", which is defined as "the application of integrated multiple-purpose design, planning and management which includes joint consideration of ground and surface water, conservational and other measures for engineering of demand, and the treatment and management of water having a substandard quality". This report has been prepared under the direction of Dr. Edward A. Ackerman.

Finally, in Committee Print No. 32, we find a summary report on the estimated supply and demand for Water in the United States for 1980 and 2000 by "Resources for the Future". The basic projections and information supplied by the Federal agencies for each of the 22 regions into which the contiguous parts of the United States were divided for the purposes of the study has enabled tentative balance sheets of water supply and demand to be drawn up. The report also deals with the basic physical problems of providing the necessary facilities for storage and treatment of water. It shows very clearly the great economic advantages that could follow from the more complete treatment of wastes.

These reports, together with the hearings, occupy some 5,800 pages and provide a most detailed picture of the present practice of use of water in the United States, and of impending problems. The final report of the Select Committee on National Water Resources to the Senate (No. 29 of the Eighth-seventh Congress, First Session) summarizes the findings and gives the Committee's conclusions and recommendations. The need for meeting problems before they arise is stressed, and five major categories of effort are thought desirable so as not to inhibit national or regional economic growth: (*a*) regulating stream-flow through the construction of surface reservoirs and watershed management; (*b*) improving the quality of streams through pollution abatement programmes; (*c*) making better use of underground storage; (*d*) increasing the efficiency with which water is used through the elimination of wasteful practices, improved sewage treatment methods, recirculation, increased irrigation efficiency, and substitution of air for water cooling; (*e*) increasing the natural yield of water by desalting, weather modification, and other artificial means.

The Committee's main recommendations to Congress are accordingly:

(1) The Federal Government, in co-operation with the States, should prepare, and keep up to date, plans for comprehensive water development and management for all major river basins in the United States. The Executive Branch should be requested to submit plans to Congress in January 1962, for undertaking and completing such work in all basins by 1970.

(2) The Federal Government should stimulate more active participation by the States in planning and undertaking water development and management activities by setting up a 10-year programme of grants for water resources planning.

(3) The Federal Government should undertake a co-ordinated scientific research programme on water. This should include both research into ways to increase available supplies and ways to increase efficiency in the use of water. The Executive Branch should be requested to submit a programme of research to Congress in January 1962.

(4) The Federal Government should prepare biennially an assessment of the water-supply demand outlook for each of the water resource regions of the United States. The Executive Branch should be requested to submit a programme of research to Congress in January 1962.

(5) The Federal Government, in co-operation with the States, should encourage efficiency in water development and use, by regulating flood plain use, by studying and anticipating water problems, by anticipating storage needs, by increasing public awareness of water problems and by the provision of public hearings where necessary.

Water is to-day assuming an ever-increasing importance as civilization's needs for water and water-related products and services grow. Areas of the globe that will thrive, or even survive, will be determined by the availability of water resources and their wise use to serve man. Growing communities will need increasingly to organize and conserve these resources. Commendable foresight has been shown in the United States in the efforts being made to provide adequate water supplies for the next generation. Most countries, especially the United Kingdom and many members of the Commonwealth, will find the approach, the reports, the findings and the recommendations of the Senate Select Committee which have been considered here of great value and interest to them in dealing with their own water problems.

W. G. V. Balchin

FLORA EUROPÆA

The second symposium of Flora Europæa was held during May 21–28, 1961, in Italy. It was divided into three parts, the first in Genoa, where five days were devoted to various papers and discussions, the second consisting of an excursion to Viareggio and the Apuane Alps, ending in Florence where the final sessions of the symposium were held in the Botanical Institute.

The meeting was attended by participants from Denmark, Switzerland, Sweden, Finland, Iceland, Poland, Portugal, Spain, Rumania, Germany, Yugoslavia, Belgium, France, Austria, Norway, as well as Italy and by all the members of the British Editorial Committee.

In Genoa the symposium was held in the University Botanical Institute, founded by the British philanthropist and merchant, Thomas Hanbury, whose name the Institute still bears. The Institute is situated high above the city, surrounded by its Botanic Garden, and made a very appropriate and comfortable meeting place. The arrangements were made by Prof. R. E. G. Pichi-Sermolli, director of the

Institute, with the co-operation of the University authorities.

The keynote of the meeting was international co-operation in European taxonomy, an appropriate theme for the Flora Europæa organization.

The opening session on Sunday morning included speeches of welcome by Prof. Pichi-Sermolli and University dignitaries, to which Prof. T. G. Tutin (Leicester, chairman of the Editorial Committee) replied in English and Prof. D. A. Webb (Dublin) in Italian. In the afternoon Prof. Tutin outlined the progress made by the Flora Europæa organization since the last meeting in Vienna. He recalled how the Editorial Committee had received a substantial grant from the Department of Scientific and Industrial Research, which enabled the secretariat to be established on a permanent basis and allowed the employment of two research assistants. The circulation of manuscripts of Volume 1 to the regional advisers had proceeded successfully during the past two years, and he discussed the three main points that had been raised by the advisers. These were distributional, ecological and cytological data. There had been great insistence on the importance of giving as detailed ecological information as possible, but in the discussion that followed Prof. Tutin's paper it was made quite clear that although some species have a fairly consistent general ecology there are many which show surprising exceptions or unexpected variation. None the less some advisers, such as Prof. R. Nordhagen (Oslo), felt that even such elementary information as whether a species grew in wet or dry habitats should be given. Prof. W. Lüdi (Zurich) suggested that certain generalized information could be given by using categorized types of ecological preferences which would be useful for non-taxonomists. It was eventually agreed that authors of accounts should attempt to summarize the ecological characteristics of the species in the manuscripts circulated to the regional advisers and these could then be criticized by them.

Dr. V. H. Heywood (Liverpool, secretary of the Editorial Committee) then gave a brief report on the state of preparation of Volume 1 of the *Flora*. The first manuscripts were sent out to regional advisers in December 1958. Since then 150 have been circulated and a further 50 have been prepared and are being revised by the Editorial Committee prior to circulation. Approximately two-thirds of the first volume have been completed and manuscripts of the remaining third are in active preparation. Furthermore, several authors are preparing accounts for the second volume and some have already been submitted. Since the Vienna meeting a supplement to the Green Book (*Guide to Contributors*) had been published[1], the Vienna Symposium report had appeared as a number of *Feddes Repertorium*[2] and the first of the *Flora Europæa Notulæ Systematicæ* were in the press[3].

The second day of the meeting was devoted to a consideration of post-war European taxonomic and floristic literature. Regional advisers were asked before the meeting to prepare reports on the situation in their countries, together with a bibliographical list of the more important papers published since 1945. Reports from 16 countries were received and duplicated copies were distributed to participants as a volume of some 250 pages.

Dr. Heywood gave an introductory analysis of these floristic reports, making the following points: It was interesting to note how frequently it was mentioned that taxonomic and floristic studies had been neglected in several countries since the War for one reason or another. Equally interesting were the reasons for renewal of activity in many cases. For example, phytosociological or topographic-distributional investigations (often undertaken for economic reasons) have given an impetus for much floristic work. There were dangers, however, attendant on this since it often involved cursory identification in the field subject to considerable errors. It was in fact true of certain regions that the only floristic information available had to be drawn from phytosociological lists. Another noteworthy feature was the lack of modern Floras in the Mediterranean region, precisely those areas with the greatest floristic diversity. In more northern countries the tendency was towards biosystematic studies. An unfortunate feature was the small number of monographs or revisions that were being prepared, a tendency which fits in with the world pattern.

The number of journals in which European taxonomic and floristic literature is published is about 500, which in itself presents problems, since few institutions regularly receive more than a proportion of them. As a result there is often considerable ignorance as to the activities of other taxonomists, with consequent duplication of effort in some cases. The solution to this kind of problem lies very largely in improving abstracting services, a subject which was discussed later. It was encouraging to note that several European journals or organizations already produce some sort of annual listing of relevant literature published in their country. Prof. Böcher (Copenhagen) suggested that it would be of great service to taxonomy if these annual reports could be united and circulated together. It was agreed to investigate the possibilities of doing this.

Brief surveys of post-war taxonomic activity were then given by Prof. T. W. Böcher (Copenhagen, for Denmark), Prof. F. Markgraf (Zürich, for Albania), Prof. R. Nordhagen (Oslo, for Norway), Dr. A. Lawalrée (Brussels, for Belgium), Prof. A. Borza (Cluj, for Rumania), Prof. K. H. Rechinger (Vienna, for Austria and Greece), Dr. N. Hylander (Uppsala, for Sweden), Prof. R. E. G. Pichi-Sermolli (Genoa, for Italy), Dr. S. M. Walters (Cambridge, for Great Britain), Prof. D. A. Webb (Dublin, for Ireland), Prof. B. Pawlowski (Cracow, for Poland), Prof. W. Lüdi (Zürich, for Switzerland), Dr. A. Löve (Montreal, for Iceland), Dr. Pinto da Silva (Sacavém, for Portugal), Prof. A. R. Merxmüller (Munich, for West Germany), Prof. W. Rothmaler (Griefswald, for East Germany), Prof. E. Guinea (Madrid, for Spain), Dr. P. Jovet (Paris, for France), and Prof. E. Mayer (Ljubljana, for Yugoslavia).

It is intended to publish revised versions of all these floristic reports in a special number of the journal *Webbia* in 1962.

On May 23, two papers were given on the Italian flora. Dr. G. Moggi (Florence) gave a survey of the state of floristic investigation in the southern half of Italy and in Sicily. It was a revelation to most participants to learn just how poorly large areas of southern Italy had been studied floristically, and it is hoped that Dr. Moggi's map illustrating this will be published with his paper as part of the *Proceedings of the Symposium*. The second paper was by Dr. S. Pignatti (Padua) on the taxonomy and relationship of the genus *Limonium* in Italy and Spain. This was followed by a lively discussion.

XUM

The following morning was devoted to considering the problems of abstracting and indexing European taxonomic literature. Dr. S. M. Walters (Cambridge) surveyed the major schemes of indexing and abstracting already in existence, such as the *Index Kewensis*, *Excerpta Botanica*, and national ones such as the *Plant Breeding Abstracts* published by the Commonwealth Bureau of Plant Breeding and Genetics, Cambridge, and certain private systems. All these have certain limitations such as incompleteness. The second question to be considered was how far they overlapped one another, and the third aspect covered was a consideration of how these facilities could be improved and modified. During the discussion attention was directed to several other abstracting systems that existed, and the regional advisers agreed to submit information on these to the Flora Europæa secretariat. The combined information would then be circulated.

A more long-term possibility of improving the abstracting facilities was discussed by Mr. S. Gould (Connecticut) who attended the meeting as an observer. He is working with a grant from the U.S. National Science Foundation, towards the setting up of an International Plant Index. The details of his methods, which involve the use of a punched card system, were explained and keenly discussed, and many participants agreed that the International Plant Index might form a useful basis which could be utilized for indexing European taxonomic literature. The possibilities of co-ordination would be further investigated and it was hoped that Mr. Gould's scheme would be successfully continued.

The next paper was by Dr. E. Weinert (Halle), who gave, on behalf of Prof. H. Meusel (Halle), who was unable to attend, examples of methods of indicating extra-European distributions for species in the text of *Flora Europæa*. The general principles employed in the examples were accepted, and Dr. Weinert said that the staff of Prof. Meusel's Institute would supply the necessary information for the whole of Volume 1, details in critical cases to be arranged with the authors of the accounts.

A problem related to the use of European taxonomic literature was reported on by Prof. D. A. Webb, who outlined progress that had been made in the preparation of a *Lexicon Polyglottum*. This was essentially a Latin-based list of about 200 terms commonly used in Floras with the equivalents in most European languages. A number of partial lists had already been prepared for immediate use and these had revealed a number of unexpected problems. It was, for example, impossible to find equivalents for certain terms in some languages. Also, terms may be used in different senses in some countries so that considerable problems of interpretation arise in producing the glossary.

The question of terminology was then discussed further by Prof. N. A. Burges (Liverpool), who outlined the problems he had come across in attempts to introduce some sort of standardization of terminology in the manuscripts of the first volume of the *Flora*. As might be expected, this generated a lively controversy over the use and application of technical terms. What several participants regarded as loose British usage of the term 'fruit' came in for considerable criticism and several minutes were devoted to discussing how the fruiting structures of *Ranunculus* and similar genera should be described. The discussion had to be terminated before it extended to detailed consideration of the whole botanical vocabulary. Prof. Burges suggested that the number of complicated terms used was to some extent correlated with the age and experience of the writers of the manuscripts, although there were exceptions, but this provocative issue was not pursued!

Alien plants was the first subject for discussion on May 25. Prof. Webb recalled the various suggestions that had been made at the Vienna Symposium concerning the treatment of aliens, notably by Dr. Hylander (Uppsala) and Prof. Jalas (Helsinki), and outlined the decisions made by the Editorial Committee. Several of the proposals, such as the distinction of casual species by a special sign, had been rejected on the grounds that the number of species involved would be too large, to say nothing of the difficulties of obtaining the necessary information. Similarly the proposal to include archæosynanthropes with native plants and the designation of only neosynanthropes as alien was rejected on the grounds of impracticability. Other proposals were however accepted, such as the inclusion of well-established aliens even if found only in one small district. Prof. Webb then went on to consider the terminology to be applied since there was considerable confusion in the literature. The term 'adventive', for example, is used in various senses. The terms proposed for use in *Flora Europæa* were: (1) cultivated or planted; (2) wild; (3) native; (4) alien; (5) established (or naturalized) alien; (6) casual. Definitions of these were given, and regional advisers were invited to submit agreed equivalents in their own languages. Criteria for the inclusion of aliens in *Flora Europæa* were then discussed. Prof. Webb concluded with a call for information about: (*a*) botanists who take a special interest in alien plants and who could assist in assessing information about them according to the criteria agreed on; (*b*) literature references published since the appearance of the standard Flora of the country. This information was particularly urgent for a number of countries such as Albania, Corsica, Greece, Spain, Sardinia, Turkey-in-Europe, none of which possesses an adequate Flora less than twenty years old.

The next subject for discussion was again introduced by Prof. Webb. It concerned the need for the preparation of regional check-lists for *Flora Europæa*. In a number of regions the information available in existing Floras was not sufficient to establish the presence even of non-critical species. The compilation of lists for certain regions such as Albania (P. W. Ball, Liverpool), Turkey-in-Europe (D. A. Webb, Dublin), Spain (V. H. Heywood, Liverpool) was now in hand, but lists for such areas as Greek Macedonia and Thrace, and Yugoslav Macedonia were urgently needed. Another such area was Sardinia, and Prof. Pichi-Sermolli thought that it might be possible to organize a list.

The final session in Genoa was devoted to a consideration of hybrids in *Flora Europæa*, and the main paper was given by Prof. D. H. Valentine (Durham) who, after a theoretical introduction, surveyed the type of problems arising in genera such as *Rumex*, *Salix* and *Saxifraga* which occur in the first volume of the *Flora*. No major departure from the rules set out in the *Guide to Contributors* was proposed, although there were proposals for greater consistency in the designation of hybrids by formulæ and binomials.

In the discussion attention was directed to the necessity for careful investigation in groups where hybridization was known, since the hybrids often resembled one of the parents so closely that they were

not detected. The question of intergeneric hybrids was also discussed, particularly with reference to the Gramineae.

In Genoa both the University and the city authorities provided generous hospitality. A reception was held at the Palazzo Tursi by the Mayor of Genoa, and the participants had an opportunity of examining there some of the original correspondence of Christopher Columbus. An extensive tour of Genoa and the Riviera de Levante, including Santa Margherita Ligure and Portofino, was provided by the Tourist Organization. The meeting in Genoa ended with a banquet given by the University at Boccadasse. The festivity of this occasion was enhanced by several speeches, including one by Prof. A. Borza in Latin which received an ovation. Prof. Borza, the doyen of the botanists present, took the opportunity of announcing two new species he had discovered—*Tutinia gravis* and *Heywoodia agilis*. Since the eloquent Latin descriptions he gave were only verbal and were not therefore effectively published according to the *International Code of Nomenclature*, steps are being taken to remedy this through other channels.

The next two days were spent on a profitable and enjoyable field excursion—on May 26 to the nature reserve of Portofino, Passo del Bracco and the celebrated *Pinus pinea* forest at Migliarino, spending the night at Viareggio. On May 27 the excursion went inland to the marble mountains of the Apuane Alps, climbing to the peak of Monte Altissimo. Florence was reached by evening and the final sessions of the symposium were held the next day, May 28, in the Botanical Institute of the University. In the morning members were shown around the various sections of the Institute, including the newly constructed phytotron and the elegant and spacious herbaria and library. A reception was held in the library dominated by the bust of Philip Barker Webb, whose herbarium, together with the other major collections, make Florence one of the most important centres for taxonomic botany in Europe.

The closing session of the symposium was held in the afternoon when the main business was the report of the Nomenclature Sub-committee, given by the chairman, Dr. N. Hylander. Most of the discussion centred round the validity of publication of the species contained in such works as Gandoger's *Flora Europae*. It was decided to make further studies and arrive at an assessment of the number of species involved and the number of changes of name which they might cause before reaching a decision. It was also agreed to circulate lists of *nomina ambigua* to the members of Flora Europæa with the view of reaching agreement on which of these names should be rejected.

The meeting ended with speeches by Prof. Pichi-Sermolli and Prof. Lüdi. Prof. Borza expressed the hope that it would be possible to hold the next meeting in Rumania. The second Flora Europæa symposium was thus successfully concluded, and co-operation for the future firmly established.

V. H. Heywood

[1] Heywood, V. H. (compiler), *The Presentation of Taxonomic Information, Supplement* (Alcobaça, 1960).
[2] Heywood, V. H. (edit.), *Feddes Repertorium*, **63**, Heft 2 (1960).
[3] Heywood, V. H. (edit.), *Feddes Repertorium*, **64**, Heft 1, 1 (1961).

EUROPEAN BROADCASTING CONFERENCE, STOCKHOLM

DURING May 26–June 23, a European conference on "Broadcasting" was held in Stockholm under the auspices of the International Telecommunication Union. The objects of the conference were: first, to revise the 1952 Agreement reached also at Stockholm for the use of the very high-frequency Bands I, II and III for sound and television broadcasting; and secondly, to prepare plans for the future development in the European area of sound broadcasting in Band II, and of television in the ultra-high frequency Bands IV and V. This year the conference was attended by delegates from some forty national administrations, and by representatives of a number of international organizations.

The conference was opened by Dr. Hakan Sterky, director-general of Swedish Telecommunications; and at the first meeting, Dr. E. Esping, technical director of Swedish Telecommunications, was appointed chairman, assisted as vice-chairmen by Captain C. F. Booth and Mr. A. Badalov, leaders of the United Kingdom and U.S.S.R. delegations, respectively.

The technical basis for this recent conference had been formulated in March at a meeting of technical experts of the International Radio Consultative Committee held in Cannes. A detailed report of this meeting provided full information on technical matters, such as the characteristics of transmitters and antenna systems, propagation curves for land and sea conditions, and the signal protection ratios requisite for satisfactory reception of television in the ultra-high-frequency bands from 470 Mc./s. upwards.

The final report adopted by the recent conference at Stockholm will await endorsement by the various national administrations represented. It contains full details of the stations proposed for sound and television broadcasting in the future. In particular, some five thousand stations are listed in the ultra-high-frequency Bands IV and V, together with the frequencies assigned to these stations to avoid mutual interference during operation. A uniform channel-width of 8 Mc./s. was proposed for each station, consistent with the adoption of the 625-line standard for the vision signals. In view of the rapid development of television throughout the world at the present time, it was very opportune that this conference was held so that satisfactory plans could be made on a sound technical basis.

One of the participating bodies at Stockholm was the Inter-Union Committee for the Allocation of Frequencies for Radioastronomy and Space Science, which was established by the International Council of Scientific Unions last year. This committee was represented by Drs. H. Sterky, and J. H. D. von der Toorn, by Prof. B. Linblad, and by Dr. R. L. Smith-Rose, secretary-general of the Committee; and it put forward two recommendations designed to protect observations made by radio astronomers in certain frequency bands. One of these sought to secure the free use of the band 606–614 Mc./s. for the radio astronomy service, and was received very sym-

XUM

pathetically by the delegates present at Stockholm, who in a majority of cases agreed to avoid, so far as is practicable, the use of this band for television broadcasting. The second recommendation pointed out the danger of harmonics from certain broadcasting stations in Bands IV and V interfering with radio astronomy observations in the hydrogen band 1,400–1,427 Mc./s. which, in the existing International Radio Regulations, is allocated exclusively for the radioastronomy service. It was recommended that administrations should take all practicable precautions to avoid such interference.

THE BRITISH ELECTRICAL AND ALLIED INDUSTRIES RESEARCH ASSOCIATION

THE annual report of the British Electrical and Allied Industries Research Association opens with a reference to the fact that it records the work of the Association's fortieth year.

Of the work started by the Association in its early days which has contributed substantially to the development of the electrical industry may be mentioned, particularly, research on insulation, on circuit breaking and switchgear, and on the loading capacity of cables. These branches of work still feature largely in the Association's activities.

It was in the third year of the Association's existence that electricity supply undertakings, joining as associate members, initiated the co-operation in research between manufacturers and supply authorities which has continued ever since. Work which is of vital importance to the supply industry and which has been pursued continuously for many years is the determination of the properties of steam at high temperatures and of the characteristics of steel in relation to withstanding high temperatures and stresses.

The present major classifications of the Association's research activities are as follows: insulation and dielectrics; switch and control gear; power plant; insulated cables; overhead lines; surge phenomena; integrating meters; magnetic materials; transformers; flame-proofness and intrinsic safety; earthing; safety and circuit problems; rural electrification; heating, cooking and allied problems; electric equipment of automotive systems and communication interference. In addition, there is a small miscellaneous group designated "Unclassified Researches".

The more important results obtained during the year in these various fields, summarized in individual paragraphs of the report, comprise for the most part technological information which is applicable directly in the electrical industry, but there is in addition a substantial volume of new scientific knowledge.

An investigation of the resistance of industrial insulating materials to surface discharges, a phenomenon known as 'tracking', has shown that the resistance to tracking of a poor material is not increased greatly by a substantial increase in the creepage distance.

Measurements relating the temperature of buried cables to seasonal variations of soil properties have been made, and the possible advantages of using special 'backfills' for cable trenches are being investigated.

In relation to flame-proof equipment it has been shown that for some explosive gases obstructions in close proximity to, but *outside*, the flange gap increase the hazard of explosion, a wrapping of tape to improve dust tightness being particularly dangerous.

Following an investigation of the characteristics of electrical floor-heating for buildings, recommendations have been issued and are now being adopted.

It is also noted that interesting records were obtained last year of the behaviour of the heating system installed for frost protection under the road surface at the Mound in Edinburgh.

Work on vacuum circuit breakers, switches and contactors continues, and it is reported that the vacuum contactor has now reached the stage of commercial development.

In relation to interference arising from industrial radio-frequency equipment, it is stated that all the information has been obtained which is necessary for the preparation of statutory regulations.

In the field of agriculture, reference is made to investigations into methods of drying grain. It appears that for continuous-flow drying the most suitable electrical technique will be infra-red heating.

Among the fundamental researches conducted in university departments under the ægis of the British Electrical and Allied Industries Research Association may be mentioned the work carried on in Liverpool on the theory of conduction in semi-conductors. It is known that the conductivity of germanium increases by several orders of magnitude at fields of 2 V./cm. and at liquid helium temperature. This phenomenon, which is often termed 'breakdown', has been shown to be quite unconnected either with instability or with destruction of the lattice. It can, in fact, be predicted theoretically on the basis of earlier work on 'hot' electrons.

Work on gas-discharge phenomena is also in progress with the Townsend ionization chamber on the measurement of ionization and attachment coefficients, and a comprehensive range of data has been obtained for carbon monoxide.

The velocity distribution of cathode particles originating from the spot of a low-current arc between copper electrodes *in vacuo* has been investigated at Oxford.

At the University of Durham experiments are in progress on collision ionization at ultra-high frequencies.

Perhaps the most noteworthy illustration of the traditional freedom of scientific inquiry occurs under the heading "Astrophysics" in the section of unclassified research work.

Originating in the important technological investigation of arc phenomena in circuit breaking, there has developed the idea of applying fundamental electrical discharge theory to problems on the macroscopic scale of astrophysics. Since 1944 several papers dealing with the development of these hypotheses have been published.

During the past year, observations have become available from astrophysical and space research which confirm predictions of this general theory in some detail.

The application of electrical discharge theory to explain a well-known type of stellar spectrum suggested the existence of spiral nebulæ on a stellar scale. Photographic evidence of the existence of such nebulæ has become available. The theory explains the general form of the planetary nebulæ and of the gas movements observed in them.

Evidence in support of another prediction has resulted from satellite observations by U.S. naval scientists. The observations of 80,000-V. X-rays associated with solar flares implies the existence of temperatures of about 100,000,000° K. in solar atmospheric electrical discharges. The prediction that such temperatures must exist in this region was made from electrical discharge theory applied to magnetic storms.

Another interesting by-product of theoretical work in a different field is a new method of measuring the surface tension of a clean surface in a liquid. This results from the mathematical solution of the problem of the equilibrium shapes of bubbles in electrical and centrifugal fields which was required in a study of the electrical breakdown of liquids, itself part of an investigation on breakdown of highly stressed impregnated dielectrics.

The most notable addition, during the year, to the Association's experimental resources has been the building and equipping of a Creep Testing Laboratory, intended to accelerate the important investigations on the creep behaviour of steels for use at temperatures above 1,050° F. (565° C.).

At the end of the year under review a major reorganization of the Association's committee structure was put into effect. Six divisional committees now guide and superintend the Association's work, replacing twenty-two sectional committees and eighty-one research committees.

An interesting reference is made to the desirability of fostering still closer relations between the Association and the universities and technical colleges. Through its extra-mural programmes the Association already maintains close contact with quite a number of departments in universities and technical colleges. To supplement this, special facilities were instituted to provide for all universities and technical colleges in the Commonwealth obtaining the published reports of the Association. A further step has now been taken in the development of this policy in that professors of electrical engineering and heads of electrical engineering departments in technical colleges have been invited to apply for associateship of the British Electrical and Allied Industries Research Association with full membership benefits.

J. GREIG

FOUR DECADES OF STATE FORESTRY IN NEW ZEALAND

THE annual report of the Director of Forestry in New Zealand for the year ended March 31, 1960, made to Sir Ernera Tirikatene, the Minister of Forests, is full of interest*. Essentially it consists of 37 appendixes in tabular form, from which progress towards various objectives can be measured, if desired. This will be the last report made by Mr. Entrican, who has just retired after long service.

The report is refreshing for its frankness on some of the mistakes made in the past. It does not disguise the difficulties encountered in the somewhat erratic progress being made towards the building up of a sound forestry practice and the creation of areas of managed forest which are expected to make important contributions to the national economy.

The State service is organized into a number of divisions which deal with management, public and private forestry, development, which comprises training and research, protection forestry, engineering work, operations, industrial operations and, finally, commercial operations.

In the past no more than lip service has been paid to the fundamental principle in forestry of sustaining the yield, and in this report we have a plain confession of neglect to adhere to that principle. "Thus", it says, "on a 2,000-acre area to be managed on a 40-year rotation, 50 acres would be planted annually, with the first 50 acres coming to maturity . . . in the forty-first year, concurrently with the completion of planting over the whole area." New Zealand speaks thus from bitter experience. The Murupara working circle in the largest planted forest there—Kaingaroa—covers 200,000 acres, the oldest portion of which is 37–47 years of age and extends to 10,000 acres only. The next age-class —27–37 years old—covers no less than 150,000 acres, the remaining 40,000 acres being of younger ages. This vast mass of 150,000 acres, composed mainly of Monterey pine (*Pinus radiata* D. Don) is described as "steadily moving towards maturity *en masse*". For various reasons it has been found impossible to cope with the tending of this large area and, in respect of other species in which there are fewer natural deaths, the growing-stock is said to be "accumulating towards stagnation". Here there is a lesson for other countries engaging in large-scale afforestation on fertile land.

New Zealand is anxiously trying to find an outlet for what is frankly called its second-grade timber as a pulp-wood, having produced an excess of certain assortments. An export trade to Australia has been established, but New Zealand openly looks ahead to supplying pulp and paper wood to the rapidly developing populations of the Asiatic countries. A strong plea is made for increasing the area of plantations of exotic conifers, even on good farming land, to produce timber for export, and it is the producer of not enough butter-fat per acre—not of mutton and wool—that is being urged to switch over to forestry. Farmers are asked to plant trees extensively, even to the extent of providing one out of the three million acres of the prescribed afforestation programme, which is expected to increase the area of planted exotic trees to 3,000,000 acres by the year 2025.

Unlike Great Britain, New Zealand still has a large area of indigenous forest. In 1840 the area there covered by these must have amounted to about two-thirds of that of the whole country. Since then they have been considerably reduced. Their treatment has followed the usual Anglo-Saxon pattern of

*New Zealand. Report of the Director of Forestry for the year ended 31 March, 1960. Pp. 115. (Wellington: Government Printer, 1960).

XUM

unplanned clearance for farming and of devastation, and exploitation goes steadily on. At one time no thought was given to its conservation as forest, and various arguments were put forward which contributed towards its neglect. Impossibility of regenerating some of the species was one which is now being proved to be without foundation. The main contributing factor was probably the ease and assumed success with which the early exotic plantations were begun. Ironically, one authority has said recently that "the indigenous forest will serve to tide over any crisis arising out of the destruction of the exotic plantations by widespread epidemics of insects and disease".

Immense areas of the natural forests on the mountainous watersheds of the major rivers have been devastated. Browsing by animals in these areas leads to loss of tree cover followed by erosion and devastating flooding in the rich settled lowland country. In a vast area of more than two million acres of ratakamaki protection forest in the South Island, disastrous results of browsing by opossums, deer and chamois are predicted. In the Waimakariri River catchment area sheep are "slowly making a desert land by close cropping of vegetative cover and exposing the soil to erosion", and threatening the city of Christchurch with possible catastrophe by floods.

The division of protection forestry of the service is responsible for the control of noxious animals, on which nearly £312,000 was spent during the year. In official operations during the year 31,494 deer, 24,830 goats and 4,920 chamois were killed and it is claimed that a bounty was paid in respect of 1,106,000 opossums destroyed.

Not all the new exotic plantations have been made on deep volcanic soils. In one forest in South Island an area of 3,500 acres was blown over by wind on a shallow soil, where, owing to rooting difficulties, the trees appear to be unable to stand after reaching a height of 50 ft. This sort of thing is also very liable to happen in Great Britain.

The New Zealand report faces up to another forestry problem, namely, that of putting timber grown in remote areas on the market. The costs have been found to be so high that they are used as a strong argument that farmers and others should grow trees nearer centres of consumption and should exploit and convert them there. This runs counter, however, to any policy which seeks to establish, or maintain, a healthy rural population.

The future of forestry in New Zealand is looking more hopeful for two main reasons. The first is that forest management, though still very backward when compared with some European countries, is being given more attention. Management plans, which are "forest regulations prescribing the application of certain sylvicultural rules, and the execution of certain works to produce a definite result", now apply to no less than 3,080,000 acres of the indigenous forest. The definition of the objects for which each forest is to be managed is a matter of vital importance and the progress made recently is heartening.

The second reason is that great stress is at last being laid on the need for research in forestry. There is now a large staff of workers so engaged, and the Forest Research Institute at Rotorua publishes its own report, which is rather voluminous, and describes work on a wide variety of subjects. It is gratifying to see that attention since 1956 has been directed to the study of the indigenous forest, which may be a dangerous concept, but is a very significant reality in New Zealand, from which very important lessons can be learnt.

THE CASE FOR A NEW PROFESSION EMBRACING SCIENCE, LIBRARIANSHIP AND FOREIGN LANGUAGES

A SURVEY*, supported by the National Science Foundation and sponsored by the Modern Language Association of America, and carried out under sub-contract with the latter Association as part of a contract with the United States Office of Education, Department of Health, Education and Welfare, argues the case for a new profession concerned with the handling of scientific information, librarianship and foreign languages. Its six chapters delineate first the characteristics of this new profession and the influences which have determined its emergence; the snares and problems of recruitment; undergraduate and graduate education in relation to the supply of such recruits; professional training; and the nature of the professional support which is required, in which connexion the authors recommend establishment of an advisory board to devise an appropriate well co-ordinated programme of courses, seminars, research groups or short-time institutes for training those already engaged in the profession or proposing to enter it. There are numerous detailed recommendations, many of which are applicable in Great Britain also, but although Messrs. L. Cohen and K. Craven lean towards the formation of a professional body, they are not explicit on the point and do not recognize the difficulties inherent in the presence of corporate bodies and individual practitioners which have, in Great Britain, considerably hindered the development of a really strong and representative professional qualifying body.

Some of the recommendations are directed towards management, for example, those relating to salary structure, status and standards. The contribution of the scientist is recognized in recommendations relating to co-operation and mutual understanding between scientists as such and those engaged in information work. Recommendations relating to undergraduate facilities stress the importance of at least two foreign languages, a broad liberal arts education and a major course in a scientific discipline for those entering on scientific information work. Establishment of a graduate school of science information leading to a master's degree is recommended as well as research leading to the establishment of a doctoral programme in this field and the growth of specialized information centres at the universities. They also recommended that technical institutions should explore the needs of industry and government for technicians in special libraries and science information centres and offer appropriate

* Science Information Personnel: The New Profession of Information Combining Science, Librarianship and Foreign Language. By Leonard Cohen and Kenneth Craven. Pp. vi+74. (New York: Science Information, P.O. Box 624, Radio City Station, 1961.) 1.50 dollars.

curricula, covering such subjects as machine searching, operation of related communication and retrieval instruments, routine indexing and coding tasks.

While the survey anticipates recognition of a more systematic and integrated approach to the organization of scientific information and the according of professional status to the scientific information specialist, the practical difficulties which the implementation of its recommendations are likely to encounter, for example, in regard to teaching staff, at least in the United Kingdom, are scarcely faced. There is also much preliminary work to be done by those already working as information officers and librarians in this field before an appropriate professional body could be formed. Nevertheless, much already exists to form the basis, and the development of the National Lending Library for Science and Technology and of the National Reference Library of Science and Information should stimulate the support and organization of research experiment and development into the nature and techniques of communication, the information requirements of scientists, the nature of technical literature sources, manual and mechanical information systems and devices, and the organization and dynamics of information facilities, which the authors of this survey both urge and expect.

DISTRIBUTION OF BRIGHTNESS IN EXTRAGALACTIC RADIO SOURCES

By A. T. MOFFET and P. MALTBY

California Institute of Technology, Pasadena

As part of a continuing programme of angular size and brightness distribution measurements, we have observed 180 radio sources with the variable spacing interferometer at the Owens Valley Radio Observatory. In this article we report our results for a group of 88 sources which have been observed with both an east–west and a north–south baseline. All observations were made at a wave-length of 31·3 cm. Some preliminary results of the east–west observations were reported by Bolton[1].

The instrument has been described by Read[2], and consists of two equatorially mounted 90-ft. paraboloids, which can be moved along railroad tracks to certain fixed observing stations. When these observations were taken, there were stations at spacings of 200 ft., 400 ft., 800 ft. and 1,600 ft. east and north of a central junction. With one antenna at the central junction and the other at each of these stations successively, a primary set of observations was made, at transit, of the apparent intensities and positions of the sources. From these observations the amplitude and phase of the complex visibility function[3] for each source in the east–west and north–south directions were deduced. A secondary set of intensity measurements was taken off the meridian at each spacing in order to obtain supplementary information about the behaviour of the visibility function at other spacings or directions. The distribution of brightness of a source is related to its visibility function by a Fourier transformation[3]. The primary observations may be considered to be somewhat the same as observing the source with two fan beams, each having dimensions of about 2 × 50 min. of arc.

With a maximum antenna spacing of 1,557 wavelengths, we were able to distinguish details of source structure larger than about 1·5′. For a source with diameter between 0·6′ and 1·5′, an estimate of the diameter was possible, and this was taken to be the width at half-brightness of the circular Gaussian model which fitted best the observed visibility function. The structure of a larger source was determined by comparing the observed portion of the visibility function with artificial visibility functions, calculated for a number of physically reasonable models.

It was found that the majority of the sources greater than 1·5′ in extent could not be interpreted as simple, symmetrical sources in which the brightness decreased monotonically from a single peak. About 10 per cent of all the sources were found to fit a model with two equal centres of emission. Of these, Cygnus *A* is the classic example. These sources are listed in Table 1.

Table 1. Sources consisting of Two Equal Components

3*C*-75	*NGC* 5128 (centre source)
3*C*-111	3*C*-310
3*C*-134	Cygnus *A*
3*C*-219	3*C*-445
3*C*-270	

The results of the interpretation are shown in Table 2. About half the well-resolved sources were asymmetrical and could be interpreted either as consisting of two unequal components or as single asymmetrical distributions. Comparing the east–west and north–south observations, we found that these sources were usually quite elongated, with a ratio of major to minor axes of $\geqslant 2:1$. The remaining one hundred sources, which we have observed only along one baseline, seem to be distributed among the various classifications in more or less the same proportions as those reported in Table 2.

Table 2. Numbers of Sources of Different Types. Diameters refer to a Circular Gaussian Model

Interpretation	No. of sources
Two equal components	9
Halo + core	4
Asymmetrical or two unequal components	26
Diameter between 1·2′ and 2·5′ in one direction; most are elongated $\geqslant 2:1$	10
Diameter < 1·2′	39

We suggest that many of the asymmetrical sources really consist of two unequal components. To obtain further proof of this, it will be necessary to take observations with a smaller effective beam-width than that used in this work. At large antenna spacings, the visibility function of a source with two well-separated, unequal components will differ markedly from that of a source with a single asymmetrical distribution of brightness. Long baseline observations made at Jodrell Bank[4] tend to support our suggestion.

We have considered the possibility that the double radio sources may be connected with double, interacting galaxies, as is the case in Cygnus *A*. A number of identifications of radio sources with optical objects has been reported[1,5,6]. Comparing these results with our own, we find that there is not a clear correlation between double radio sources and double galaxies.

XUM

We have suggested that the majority of the discrete radio sources are double, physically linked radio systems; however, it will be necessary to have more identifications with optical objects before conclusions can be drawn about the physical mechanism responsible for this phenomenon.

Details of this work will be published elsewhere. The programme of research in radio astronomy at the California Institute of Technology is supported by the United States Office of Naval Research. One of us (A. T. M.) has held a National Science Foundation pre-doctoral fellowship during the course of this work. The other (P. M.) has a Fulbright travel grant and a grant from the Norwegian Research Council for science and the humanities.

[1] Bolton, J. G., Introductory talk at the session on discrete sources at the U.R.S.I. General Assembly, London (1960).
[2] Read, R. B., *Inst. Rad. Eng. Trans. on Antennas and Propagation*, AP-9, 31 (1961).
[3] Bracewell, R. N., *Proc. Inst. Rad. Eng.*, **46**, 97 (1958).
[4] Palmer, H. P., and Brown, R. H. (private communication).
[5] Dewhirst, D. W., *Paris Symposium on Radio Astronomy*, edit. Bracewell, R. N. (Stanford Univ. Press, 1959).
[6] Mills, B. Y., *Austral. J. Phys.*, **13**, 550 (1960).

COMPARISON OF BERYLLIUM-7 AND CÆSIUM-137 RADIOACTIVITY IN GROUND-LEVEL AIR

By Dr. P. F. GUSTAFSON, M. A. KERRIGAN and S. S. BRAR

Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois

SINCE the discovery of carbon-14 produced by cosmic rays by Anderson and Libby in 1947 [1], a number of other radionuclides, also produced by cosmic-ray interactions with atmospheric constituents, have been found in the atmosphere and in the oceans. Several investigators, notably Lal[2] and Peters[3], have suggested that these naturally occurring radionuclides might prove useful in the study of atmospheric mixing and circulation. For this purpose, beryllium-7, first reported by Arnold and Al-Salih in 1956 [4], is probably the most important. The rate of production of beryllium-7 is exceeded only by that of carbon-14 and tritium. The high specific activity resulting from its 53-day half-life and the lack of dilution by stable beryllium make it an attractive choice for such work. The emission of an 0·48-MeV. γ-ray in 11 per cent of the disintegrations[5] make it fairly easy to detect beryllium-7 by means of present-day γ-ray counters. Furthermore, detectable increases in beryllium-7 attributable to nuclear weapon tests have not been found as was the case for sulphur-35 (ref. 3).

The use of isotopic concentration as a means of measuring circulation and mixing stems from the inherently higher concentration prevailing in the stratosphere. The difference in concentration between stratosphere and troposphere is due primarily to the increase in cosmic-ray flux with altitude, and the resultant increase in isotope production via cosmic-ray neutrons as a function of altitude. In addition to lower initial production, the concentration within the troposphere is further reduced by the continual removal of activity by precipitation. Until recently all production-rates were based largely upon theory, but in 1960 Lal *et al.*[6] measured the rate of production of beryllium-7 directly in oxygen (water) exposed on Mount Evans, Colorado, and found the empirical results to be only 10 per cent higher than the calculated rate.

Beryllium-7 has been measured in surface air at Argonne National Laboratory (42° N., 88° W.) since mid-1959, at which time the concentration of γ-emitting fission products in air had diminished sufficiently so that the 0·48-MeV. line from beryllium-7 was readily apparent in the γ-ray spectrum of ashed air filters. The filters consisted of '*HV*-70' (Hollingsworth–Vose) filter paper mounted on 'Sutorbilt' air pumps and operated at flow-rates of 25–30 cubic metres per hr. The '*HV*-70' paper has an efficiency of 96 per cent for particles greater than 0·25μ in diameter. Filters from 8–10 air pumps operated continuously were grouped by calendar month and partially ashed at 300° C. (total ashing was not possible due to the presence of an asbestos base) prior to γ-counting. The volume of air from which particulates were removed was of the order of 10^6 cubic metres per month. The ashed residue was compacted into a cylindrical wafer and placed on a 5 × 4 in. sodium iodide (thallium) crystal for γ-ray spectrometric analysis. Both sample and detector (consisting of the crystal and a DuMont 6265 photomultiplier tube) were enclosed in a 9-in. thick steel shield with an inner shield consisting of 1 in. of mercury in a stainless steel annulus to achieve low background conditions. Pulse analysis was implemented using an Argonne type 256 channel analyser. Suitable sources of a number of fission products were used for standardization purposes. The concentrations of cæsium-137, zirconium-95, cerium-144, antimony-125, ruthenium-106, and beryllium-7 were determined by a method utilizing a combination of simultaneous equations and successive approximations.

The concentrations of beryllium-7 and cæsium-137 expressed in curies/cubic metre of air are shown in Fig. 1. The cæsium-137 measurements extend from January 1959 to the end of 1960, whereas those for beryllium-7 start in August 1959 and are corrected for decay to the middle of the month of collection. Both radionuclides exhibit maximum concentrations during the spring of 1960, and cæsium-137 shows a similar maximum in 1959. Such maxima, in the absence of recent nuclear tests, are attributed to the disruption of the northern hemisphere tropopause in middle latitudes during the spring months, resulting in an influx of high-altitude debris, much of it of polar origin, into the lower atmosphere. A repetition of the beryllium-7 maximum in 1959 is not seen owing to lack of data prior to August 1959; however, a similar trend in beryllium-7 concentration in surface air was noted by Cruikshank, Cowper and Grummitt[7] at two sites in Canada in 1954.

Following the period of maximum concentration, the level of cæsium-137 diminishes rapidly, proceeding at a rate in accord with that ascribed to removal of tropospheric debris by precipitation (~ 30 day half-

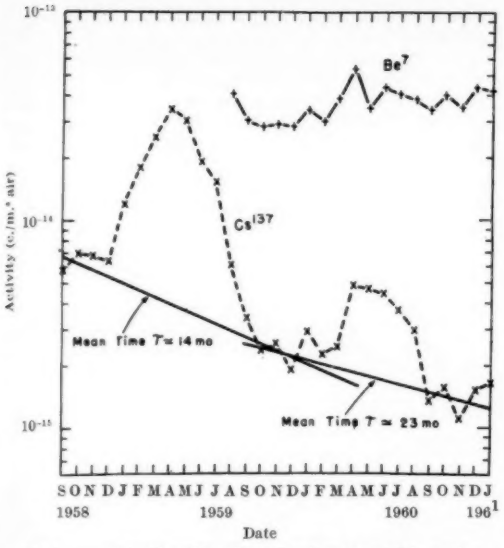


Fig. 1. Concentration of beryllium-7 and cæsium-137 in surface air at Argonne National Laboratory

time). The level of beryllium-7, on the other hand, decreased slightly and then remained at a substantially higher concentration than that observed prior to the maximum until the end of 1960. The difference in behaviour between beryllium-7 formed at a constant (though not homogeneous) rate throughout the entire atmosphere and cæsium-137 coming entirely from the stratosphere is an intriguing one, particularly in that it may provide information regarding transport of debris from the stratosphere into the troposphere. The apparent increase in beryllium-7 during the summer of 1960 might have been due to a decrease in precipitation, resulting in a lower rate of removal, and hence a build-up toward true equilibrium between production and radioactive decay. If precipitation is indeed the controlling factor, then the effect should have been in the opposite direction ; the beryllium-7 in surface air should have decreased since rainfall is more abundant during the summer months in middle and northern latitudes in the United States and Canada.

It appears that there was an actual increase in the concentration of beryllium-7 in the troposphere during the summer of 1960, and by inference during the summer each year. The basis for such a hypothesis is shown in Fig. 2, in which the reciprocal of the average monthly beryllium-7 concentration in surface air ($1/N$) is plotted as a function of the fraction of the annual precipitation which fell during the previous month (R), according to the method of Cruikshank *et al.*[7]. The assumption is made that equilibrium exists between beryllium-7 production and removal in the troposphere. Hence:

$$Q = \Lambda N \qquad (1)$$

where Q is rate of production of beryllium-7 in atoms/litre/day; Λ is rate of removal of beryllium-7 per day $= \lambda + kR$; λ is disintegration constant of beryllium-7 per day; R is fraction of annual precipitation falling in previous month; k is removal by precipitation per day; N is concentration of beryllium-7 in atoms/litre.

Equation (1) may also be written as:

$$1/N = \Lambda/Q = \lambda/Q + kR/Q \qquad (2)$$

Values of $1/N$ show a considerable range for a given value of R, the variation being outside the statistical accuracy of the determinations. For the most part the points appear to belong to two distinct groups as shown by the weighted average of each set. The intercept at $R = 0$ provides a measure of the production rate Q according to the relationship $1/N = \lambda/Q$. It should be noted that a higher concentration occurred during April–August 1960 and in August 1959. The variation in precipitation is similar during the periods of different production rate (that is, different concentration) and would seem to indicate that the difference is not due to this source. Although local precipitation values were used, they are qualitatively representative of those pertaining over a much wider area. The true effect of rainfall was probably seen during the autumn of 1960 when the beryllium-7 in surface air was consistently higher than in 1959 whereas the precipitation was markedly lower than in the autumn of 1959. The fact that the $1/N$ values fall in the low production group during this period lends support to this contention.

The question now arises as to the source of the increase in beryllium-7 concentration during the summer months. Prolonged increases in cosmic-ray intensity may be ruled out as none was observed. An increase would result if the release of beryllium-7 from the stratosphere were greater in summer than in winter. A related parameter, which varies seasonally, is the height of the tropopause in middle and northern latitudes, rising in summer and sinking to lower altitude in winter. As a result, regions of higher production of beryllium-7 will be included in the troposphere during summer and will be located within the stratosphere during the colder season. If this is the physical mechanism involved, it should be possible to verify it, in a crude way at least, by considering the change in production rate as a function of tropopause height. In the vicinity of 40° N. the mean winter tropopause is located at 33,000–35,000 ft. and that in summer at 45,000–48,000 ft. The

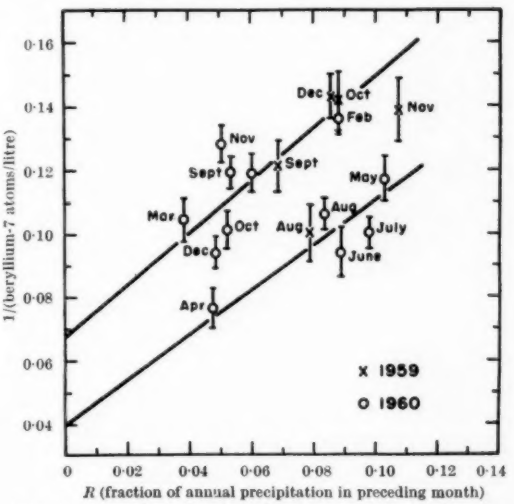


Fig. 2. Concentration of beryllium-7 in air as a function of precipitation

values of Q obtained from Fig. 2 are 0·37 and 0·20 atoms/litre/day or 260 and 130 atoms/cm.²/day for high and low production respectively. These values are a full order of magnitude lower than the value of 2,200 atoms/cm.²/day computed by Lal[2], including the empirical correction, for beryllium-7 production in the troposphere at 55° N. geomagnetic latitude. Because of inhomogeneities within the troposphere caused by incomplete mixing, removal of debris by precipitation preferentially from only the lower levels, as well as loss at the surface owing to dry deposition and impingement, measurements of air-borne debris made at ground-level are not representative of the mean level present throughout the troposphere. The existence of a fairly steep concentration gradient of air-borne debris between ground-level and the tropopause has been noted in aircraft profile studies[8]. Integration of the cæsium-137 and gross fission product concentration as a function of altitude during the spring and summer of 1960 indicated that an average factor of 13·5 existed between mean tropospheric concentration and that found at ground-level in the first half of 1960. The same factor was also found applicable to beryllium-7 concentration[9]. Converting the surface production values found from Fig. 2 into those for the mean troposphere yield values of 1,755 and 3,510 atoms/cm.²/day for winter and summer production, respectively, which are in fair agreement with that of 2,200 atoms/cm.²/day given by Lal[2]. The corresponding stratospheric production rate at this latitude computed by Lal is 9,400 atoms/cm.²/day. The mass of air present between 35,000 and 50,000 ft. is 13 per cent of the total mass in a vertical column extending from the surface to 50,000 ft. The effective mean production within the summer troposphere using this mass distribution and Lal's values for tropospheric and stratospheric beryllium-7 production is found to be :

$$Q_s = 0{\cdot}87 \times 2{,}200 + 0{\cdot}13 \times 9{,}400 = 3{,}130 \text{ atoms/cm.}^2\text{/day}$$

There is reasonably good agreement between this figure and that of 3,510 atoms/cm.²/day obtained from Fig. 2, particularly since no consideration has been given to transverse mixing of air which might further vary the apparent production of beryllium-7 as it is also a function of geomagnetic latitude.

If the concept can be accepted that the increase in concentration of beryllium-7 at the surface is due to the elevated tropopause, then it should be possible to relate the cæsium-137 concentration in the region of 35,000–50,000 ft. during and following the time of the maximum to that observed afterwards in surface air. The cæsium-137 present in the troposphere several months after a nuclear test may be considered to arise from continual leakage through the tropopause plus varying amounts of additional activity periodically injected by the spring break and the rising tropopause. The minima in levels of cæsium-137 in surface air seen in Fig. 1 during the autumn and winter are probably most closely a measure of the continual, 'steady state', removal of stratospheric debris by diffusion and minor fluctuations in the tropopause. Thus the amount of surface activity due to leakage during May–July 1960 should have been intermediate to the levels seen during late 1959 and the autumn of 1960. For May–July a concentration of 2×10^{15} curie/cubic metre was taken for cæsium-137 due to leakage (Fig. 1), which multiplied by 13·5 becomes $2{\cdot}7 \times 10^{-14}$ curie/m.³ for the mean concentration in the troposphere. Again using a value of 13 per cent for the fraction of the total air mass in the summer troposphere contained between 35,000 and 50,000 ft., and the observed value of $4{\cdot}5 \times 10^{-13}$ curie/m.³ for the cæsium-137 concentration in this region[10], a concentration of $\sim 6 \times 10^{-15}$ curie/m.³ is derived for the surface during May–July 1960. The measured concentration during this time varied from 3·8 to $4{\cdot}5 \times 10^{-15}$ curie/m.³. The essential agreement between observed and calculated values tend to support the hypothesis that the rising tropopause causes the mass transfer of debris from the lower stratosphere into the troposphere from whence it is readily removed by means of precipitation and dry deposition.

The relative importance of the rising tropopause and the spring break as they affect the total removal of stratospheric debris may be estimated by integrating the total cæsium-137 found in surface air and that presumed to come only from steady-state removal. Taking a cycle extending between successive autumn minima, it was found that from the autumn of 1958 to the autumn of 1959, 75 per cent of the removal occurred via the spring break and the rising tropopause, whereas only 40 per cent was so removed during the autumn of 1959 to autumn 1960. Of these two mass transfer processes, that of the spring break is probably more important. The greater removal during the autumn of 1958 to the autumn of 1959 is presumably due to the fact that debris from the Soviet tests in October 1958 was essentially all removed by the end of 1959. Further evidence of the importance of these perturbations to steady-state removal may be seen by considering the mean stratospheric residence-time. Since the autumn minima are believed to reflect continual steady-state removal of stratospheric debris, the relationship between successive minima, as indicated in Fig. 1, should yield information relative to the mean stratospheric residence-time. During 1958–59 the mean time so derived was approximately one year, and during 1959–60 it lengthened to more than 20 months. Hence were it not for the seasonal increases in the rate of removal of nuclear debris from the stratosphere the mean residence-time would be approximately twice what it is. The non-uniformity in the rate of stratospheric removal adds to the complexity of fall-out mechanics; however, its proper consideration and better understanding will not only improve the accuracy with which ground contamination from present stratospheric debris may be predicated, but will also further our knowledge of the meteorology of the upper atmosphere.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

We are indebted to Dr. J. Sedlet of the Industrial Hygiene and Safety Division of this Laboratory for the use of air filters from his collection network.

[1] Anderson, E. C., Libby, W. F., Weinhouse, S., Reid, A. F., Kirshenbaum A. D., and Grosse, A. V., *Science*, **105**, 576 (1947).
[2] Lal, D., Malhotra, P. K., and Peters, B., *J. Atmos. Terr. Phys.*, **12**, 306 (1958).
[3] Peters, B., *J. Atmos. Terr. Phys.*, **13**, 351 (1959).
[4] Arnold, J. R., and Ali Al-Salih, H., *Science*, **121**, 451 (1955).
[5] Strominger, D., Hollander, J. M., and Seaborg, G. T., *Rev. Mod. Phys.*, **30**, No. 2, 585 (1958).
[6] Lal, D., Arnold, J. R., and Honda, M., *Phys. Rev.*, **118**, 1626 (1960).
[7] Cruikshank, A. J., Cowper, G., and Grummitt, W. E., *Canad. J. Chem.*, **34**, 214 (1956).
[8] Peirson, D. N., Crooks, R. N., and Fisher, Miss E. M. R., *AERE-R3358* (1960).
[9] Telegadas, K., and List, R. J., Health and Safety Laboratory Quarterly Report, January 9, 1961, *HASL*-105, 150 (1961).
[10] List, R. J., and Telegadas, K., Health and Safety Laboratory Quarterly Report, April 1, 1961, *HASL*-111, 186 (1961).

SUSTAINED OSCILLATIONS IN A CATALYTIC CHEMICAL SYSTEM

By R. A. SPANGLER* and F. M. SNELL

Department of Biophysics, University of Buffalo School of Medicine, Buffalo, New York

TO account for some features of periodic phenomena observed in biological organisms, the existence of some autonomous timing mechanism, or 'living clock', has been postulated. Although many different properties of these periodic phenomena have been reported, two characteristics are of particular theoretical interest. The frequency in certain instances is virtually independent of temperature, while in a variety of species the clock appears to be subject to external synchronization and modulation[1-3]. Any theoretical consideration of a timing mechanism must be reconciled with these observations[4].

Based entirely on chemical kinetic considerations, model mechanisms which exhibit periodic behaviour may be formulated. Because of the fundamentally dissipative nature of non-equilibrium chemical processes, however, certain non-linearities of reaction kinetics must be exploited to generate undamped oscillations in a system with a finite number of variables[5,6]. As early as 1920, Lotka[7] demonstrated a theoretical basis for undamped oscillations in a sequential series of autocatalytic reactions, a class of systems which has been treated in general by Moore[8]. More recently, Holmes[9] has discussed another non-linear kinetic mechanism, but has resorted to a discontinuous reaction coupling function to achieve oscillatory behaviour. The relationship to biological reality of both these system classes would appear remote.

Using a steady-state analytical approach as well as digital and analogue computer techniques, we have undertaken a study of the periodic behaviour exhibited by a particular realistic class of catalytic reaction systems. Being a type of relaxation oscillation, the periodicity of this system is non-harmonic in nature, and sustained oscillations may be achieved over a reasonably wide range of parameter values.

Consider a cross-coupled catalytic system formulated as follows:

$$\text{source } A \rightarrow A + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B \xrightarrow{k_2} E + P \xrightarrow{k_3} \text{sink } P$$

$$\alpha P + E' \underset{k_{-4}'}{\overset{k_4'}{\rightleftarrows}} I' \qquad \alpha P' + E \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} I \qquad (1)$$

$$\text{source } A' \rightarrow A' + E' \underset{k_{-1}'}{\overset{k_1'}{\rightleftarrows}} B' \xrightarrow{k_2'} E' + P' \xrightarrow{k_3'} \text{sink } P'$$

The parameter α is the stoichiometric number for the association of the product (P or P') of one reaction sequence with the catalyst (E' or E) of the other to form an inactive complex (I' or I).

Under steady-state conditions, the following implicit relationship may be derived:

$$\left(\frac{k_3 P}{k_2 E_t - k_3 P}\right)\left(1 + K_4\left[\frac{k_1' k_2' E_t' A'}{k_3'(k_2' + k_{-1}')(1 + k_4' P^\alpha) + k_1' k_3' A'}\right]^\alpha\right) - \frac{k_1 A}{k_2 + k_{-1}} = 0$$

* Fellow, U.S. Public Health Service, Institute of Arthritis and Metabolic Diseases.

$$J_{ss} \equiv k_3 P \qquad K_4 \equiv \frac{k_4}{k_{-4}} \qquad K_4' \equiv \frac{k_4'}{k_{-4}'} \qquad (2)$$

where the species symbols are used to denote concentrations. J_{ss} is the unprimed steady-state reaction flux, defined as $k_3 P$; E_t and E_t' are the total catalyst concentrations which are conserved.

When $\alpha = 1$, the partial derivative $\left(\frac{\partial J_{ss}}{\partial A}\right)_{A'}$ remains positive and finite; thus J_{ss} is a single-valued function of A for all positive values of A. However, with $\alpha = 2$, the derivative may become negative over a limited range of A-values, passing through infinity at the boundaries of the region. J_{ss} becomes a multi-valued function of A in this region, and the form of the relationship under these conditions with A' constant appears in Fig. 1.

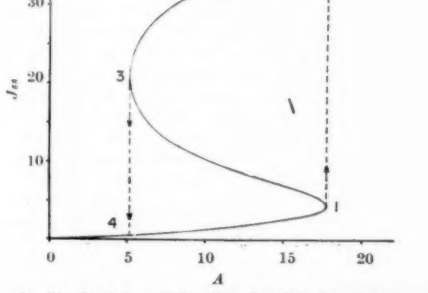


Fig. 1. Steady-state reaction flux J_{ss} plotted against reactant concentration A, as given by equation (2) with constant $A' = 10$. Axes are in arbitrary units of appropriate dimensions. Rate-constants employed for this solution are: $k_1 = k_{-1} = k_2 = k_4 = 1$; $k_3 = 0{\cdot}4$; and $k_{-4} = 0{\cdot}5$, with $k_1' = k_1$, $E_t = E_t' = 4$. Numbers on the curve are referred to in the text

It may be seen that this steady-state solution bears analogy to certain negative impedance devices, for example, the tunnel diode. Each possesses a region of negative slope, and each exhibits bi-stable, 'flip-flop' characteristics in this region. If, for example, A is varied cyclically over a range including the negative slope region, again with A' held constant, the system will proceed along the trajectory indicated by the dashed lines in Fig. 1, passing directly from point 1 to point 2 for increasing A, and from 3 to 4 as A is decreased. Thus the system exhibits what is analogous to a hysteresis loop in the phase-plot of steady-state conditions.

In the conditions that A is fixed at arbitrary concentrations, the negative slope portion of the continuous curve corresponds to possible stable steady-state solutions, although this segment is accessible only in rather physically unrealizable circumstances. On the other hand, if the influx of reactant A, J_A^n, is fixed, making A a dependent variable, this region of negative slope is characterized by instability; any random fluctuation from a given steady state in this region can only lead to

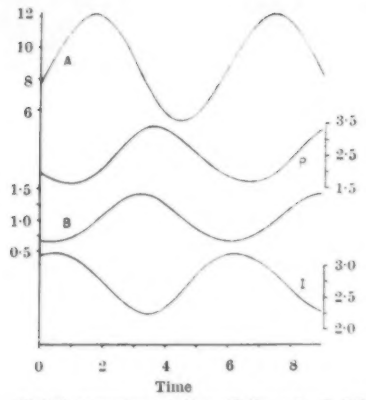


Fig. 2. Digital computer solution of the set of differential equations representing the system (1) with constant $J_A^{in} = J_{A'}^{in} = 1$. Chemical concentrations are plotted as a function of time with axes in arbitrary units. Rate-constants and the E's are the same as given in Fig. 1

greater deviation in the same direction. In these circumstances, then, with J_A^{in} between points 1 and 3 of Fig. 1, oscillations would in general be expected, since no stable steady-state solution exists which is compatible with the magnitude of flux from the source. Specifically, the system would be expected to trace in an anti-clockwise direction a closed trajectory in the phase-plot, roughly similar to the hysteresis loop, but not coinciding precisely because of departures from steady-state conditions. No general analytical solution for this dynamic situation exists, and it must be repeated that the foregoing analysis is based on the assumption of steady-state conditions and a constant A' concentration. The oscillations predicted by this analysis are evident, however, in the digital and analogue computer solutions of the dynamic system. Furthermore, oscillatory behaviour has been observed in the computer solutions of the fully symmetrical system in which both J_A^{in} and $J_{A'}^{in}$ are fixed. An example of these oscillations may be seen in Fig. 2.

Further work is in progress on this and other classes of reactions in an effort to delineate the detailed conditions for sustained oscillation. Preliminary observations, however, suggest several general properties of this particular system. It would appear, for example, that the frequency of oscillation of the complete system is less dependent, thermally, than the individual reactions, and it is conceivable that complete temperature compensation may be obtained by having appropriate relationships among temperature coefficients of the rate constants. It also appears that the period of oscillation is one-to-several orders of magnitude larger than the time-constants of the individual uncoupled catalytic reactions, a feature possibly of significance in consideration of the long periods of some biological phenomena. In addition, several means by which external stimuli may modulate the phase or frequency of oscillation suggest themselves, such as by alteration of either the reactant influx or the total catalyst concentration.

In view of these preliminary results and the feasibility of the assumptions involved, it appears that biological periodicity may be based entirely on chemical kinetic systems, without resort to diffusive or phase boundary effects. The model discussed here is proposed as one specific mechanism possibly underlying the biological clock.

We are grateful to Mr. Harold Meese and Dr. John Fleck of the Cornell Aeronautical Laboratories for their assistance and the use of the *IBM* 704 computer facilities. This investigation was supported by a U.S. Public Health Service Research Grant No. *RG*–6730 from the Division of General Medical Sciences.

[1] Bünning, E., *Cold Spring Harbor Symp. on Quantitative Biology*, **25** (1960).
[2] Pittendrigh, C. S., *Cold Spring Harbor Symp. on Quantitative Biology*, **25** (1960).
[3] Brown, F. A., *Science*, **130**, 1535 (1959).
[4] Schmitt, O. H., *Cold Spring Harbor Symp. on Quantitative Biology*, **25** (1960).
[5] Hearon, J. Z., *Bull. Math. Biophys.*, **15**, 121 (1953).
[6] Finn, R. K., *Bull. Math. Biophys.*, **16**, 181 (1954).
[7] Lotka, A. J., *J. Amer. Chem. Soc.*, **42**, 1595 (1920).
[8] Moore, M. J., *Trans. Farad. Soc.*, **45**, 1098 (1949).
[9] Holmes, W. F., Abstr. Fifth Ann. Meeting of the Biophys. Soc., St. Louis, Missouri, TB 13, February 1961.

MINIMUM ENERGY THEOREM FOR FLOW OF DRY GRANULES THROUGH APERTURES

By R. L. BROWN
British Coal Utilization Research Association, Leatherhead, Surrey

ALTHOUGH the motion of granules flowing down a vessel to escape through an aperture is complex, the flow-rate (Q gm./sec.) over small intervals of time is remarkably steady. It seems permissible to represent the average flow by stream-tubes in a continuum of density ρ gm./c.c. Then conservation of number flowing in a stream-tube gives:

$$N_1 dA_1 = N_2 dA_2 \qquad (1)$$

where N granules per cm.²/sec. are flowing orthogonally to an elementary area dA (Fig. 1). If v cm./sec. is the velocity of the continuum normal to dA:

$$\rho v = mN \qquad (2)$$

where m gm. is the weight of a granule. It is assumed that the granules all have the same weight.

Consider an elementary volume $d\tau$ c.c. at co-ordinates (R, θ). The mass is $\rho d\tau$ gm., the height above the origin of co-ordinates is $R \cos \theta$ cm. and the velocity v cm./sec. Writing $T d\tau$ for the sum of the potential and kinetic energies:

$$2T = \rho(v^2 + 2gR \cos \theta) \qquad (3)$$

Now it is well known[1] that the flow-rate Q does not vary with the head of material in the vessel, except perhaps at very small heads. Pressure is not transmitted far in a granular mass, owing to the development of arches and to frictional forces between granules. These considerations suggest that the pressures on the faces dA of the volume $d\tau$ do little or no work. Energy is dissipated, however, on the

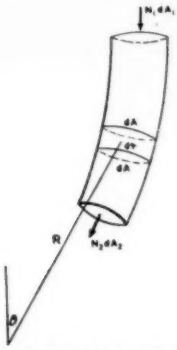


Fig. 1. Stream-tube in continuum

curved surface of $d\tau$ and by collisions, rotations and frictional forces within $d\tau$. It is postulated therefore that the energy T per unit volume decreases in the direction of flow along a stream-tube. Remembering that the granules below the aperture are falling freely (apart from collisions in horizontal planes), the vertical pressure just above the aperture cannot be large, and it seems likely that the energy postulate will hold at least in the neighbourhood of the aperture, provided that there is no appreciable cohesion between granules.

Above apertures in a horizontal base plate, a portion of the mass resting on the base is stationary, and the stream-tubes are forced to converge. Visual observation suggests that, to the first order, the flow takes place in a straight-sided wedge or cone with half-angle β (Fig. 2). The simplest way of satisfying the continuity equation (1) is to take the stream-tubes to be radial. Then for plane flow between parallel vertical plates, distant l cm. apart, through a slit of width S cm.:

$$N = \frac{AK(\theta)}{R} \tag{4}$$

whence, taking A independent of (R,θ),

$$Q = 2mAl\int_0^\beta K(\theta)\mathrm{d}\theta \tag{5}$$

For axial flow down a cylinder through a circular aperture of diameter D cm.:

$$N = \frac{BL(\theta)}{R^2} \tag{6}$$

and

$$Q = 2\pi mB\int_0^\beta L(\theta)\sin\theta\mathrm{d}\theta \tag{7}$$

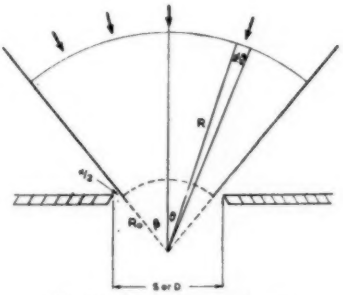


Fig. 2. Flow in a wedge or cone

It has been shown elsewhere[2] that there is an effectively empty annulus, of width $k/2$ cm., adjacent to the edge of the aperture. k is the same for slits and circles and depends *inter alia* on the size of the granules. This annulus can be taken into account (Fig. 2) by locating the vertex of the wedge or cone a distance R_0 cm. from the edge of the annulus, where:

$$2R_0\sin\beta = (S - k) \text{ or } (D - k) \tag{8}$$

In what follows it will be assumed that ρ is constant throughout the wedge or cone, apart from a possible discontinuity at $\theta = \beta$. Thus v is taken to be proportional to N.

Then in plane flow, equations (2), (3) and (4) give:

$$2T = \rho\left[\frac{m^2A^2K^2(\theta)}{\rho^2R^2} + 2gR\cos\theta\right] \tag{9}$$

which has a minimum at R_m where:

$$gR_m^3 = \frac{m^2A^2K^2(\theta)}{\rho^2\cos\theta} \tag{10}$$

Likewise for cylindrical flow, equations (2), (3) and (6) give:

$$gR_m^5 = \frac{2m^2B^2L^2(\theta)}{\rho^2\cos\theta} \tag{11}$$

If the energy postulate that T decreases along a stream-tube is to hold, then the surface of minimum energy (R_m,θ) must lie below the wedge or cone.

The flow-rate Q may be expressed in terms of aperture size and the surface of minimum energy by means of equations (5), (7), (8), (10) and (11), namely:

$$\sigma = \frac{Q}{\rho l(S-k)\{g(S-k)\}^{1/2}} = \frac{\int_0^\beta (R_m/R_0)^{3/2}\cos^{1/2}\theta\mathrm{d}\theta}{2^{1/2}\sin^{3/2}\beta} \tag{12}$$

$$\gamma = \frac{4Q}{\rho\pi(D-k)^2\{g(D-k)\}^{1/2}} = \frac{\int_0^\beta (R_m/R_0)^{5/2}\cos^{1/2}\theta\sin\theta\mathrm{d}\theta}{\sin^{5/2}\beta} \tag{13}$$

It would not be unexpected in flow from wide vessels to have β a constant, determined by material characteristics. If this were the case, equations (12) and (13) show that σ, γ are constants, provided (R_m/R_0) is a function of θ,β only. Experimental evidence has been given elsewhere showing that σ, γ are constants for several materials discharging from wide vessels through a range of aperture sizes[2]. Such discrepancies as have been noted, are small. (This is not the case for narrow vessels.) It would appear, therefore, that (R_m/R_0) is, to the first order, a function of θ,β only.

Now the sides of the wedge or cone at $\theta = \beta$ cease for $R \ll R_0$. Thus the surface $R = R_0$ can be regarded as an upper limit to the surface (R_m, θ) whence equations (12) and (13) yield upper limits for σ, γ:

$$\sigma_u = \frac{\int_0^\beta \cos^{1/2}\theta\mathrm{d}\theta}{2^{1/2}\sin^{3/2}\beta} \tag{14}$$

$$\gamma_u = \frac{2(1-\cos^{3/2}\beta)}{3\sin^{5/2}\beta} \tag{15}$$

It is interesting to note that, taking the average velocity around the arc of the wedge as $(Q/2R\beta l\rho)$ and

Table 1. Flow from Flat-bottomed Vessels

Material	Symbol	Mean weight diam. (cm.)	Empty annulus (k cm.)	Bulk density† (ρ gm./c.c)	Plane flow		Cylindrical flow	
					β degrees	observed σ	β degrees	observed γ
Glass beads	(1)	0·096	0·13	1·71	65	0·82	31–34	0·69
Glass beads	(2)	0·023	0·041	1·70	66	1·16	40–41	0·83
Rounded sand	X	0·062	0·10	1·49	52	0·82	27–30	0·68
Rounded sand	●	—	—	1·50*	56	0·90	—	—
Tapioca	T	0·145	0·22	0·72	56	0·82	29–31	0·68
Sharp sand	1	0·054	0·12	1·25	41	0·90	29–30	0·74
Sharp sand	2	0·038	0·079	1·23	45	0·95	22–27	0·75
Sharp sand	3	0·017	0·036	1·18	40	0·87	22–26	0·71
Coal	C	0·061	0·14	0·65	45	0·90	32–35	0·72

* Assumed.
† A minimum value measured by fluidizing bed and then slowly reducing air flow to zero; used for ρ in equations 12 and 13.

the average height of the arc as $(R \sin \beta/\beta)$, application of the minimum energy theorem at $R_m = R_0$ gives:

$$\sigma_{av.} = \frac{\beta^{1/2}}{2^{1/2} \sin \beta} \qquad (16)$$

the corresponding equation for cylindrical flow being:

$$\gamma_{av.} = \frac{(1 - \cos \beta)^{1/2}}{2^{1/2} \sin^{3/2} \beta} \qquad (17)$$

For values of β ranging to more than 70°, equations (16) and (17) differ by less than 1 per cent from equations (14) and (15), respectively. It would seem, therefore, that the flow-rates may not be very sensitive to the profiles $K(\theta)$, $L(\theta)$. These profiles are associated with the minimum surface $(R_m.\theta)$ by equations (10) and (11).

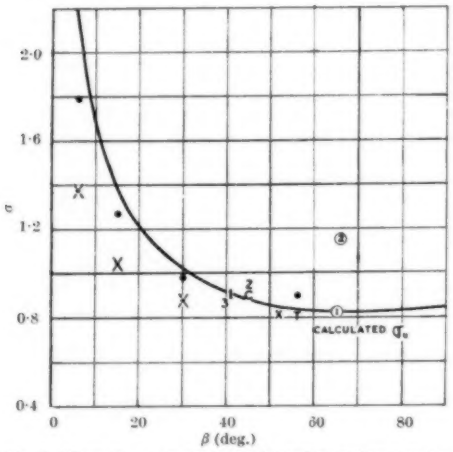


Fig. 3. Plane flow. Symbols are defined in Tables 1 and 2

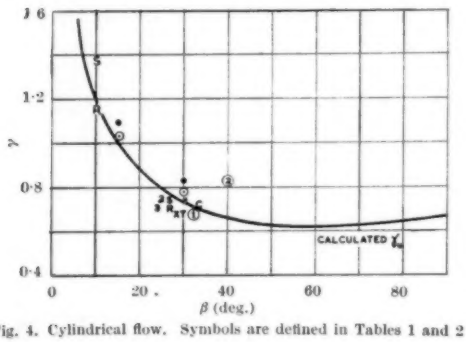


Fig. 4. Cylindrical flow. Symbols are defined in Tables 1 and 2

Data already published on σ,γ can be used to test whether the predicted relation of σ_u,γ_u with β, is valid. This is attempted first for flow from flat-bottomed vessels where the material forms its own wedge or cone. The angle β in plane flow was measured by observation through a 'Perspex' side plate. Marker particles were used in cylindrical flow; those emerging in the first runnings were noted. The estimates of β found by these methods are considered to be reasonably good; details will be given elsewhere. It was found that β for cylindrical flow was appreciably less than for plane flow (Table 1).

The observed values of σ,γ are plotted against β in Figs. 3 and 4. Agreement with the calculated values of σ_u,γ_u is seen to be good and it follows that the angle β of the wedge or cone is a major factor in determining the rate of efflux. It is satisfactory to see that in plane flow the experimental data follow the theoretical curve round to its minimum at about 65°. At larger values of β, flow would be taking place at angles below the angle of rest to the horizontal, which is clearly impossible. The theoretical equation for cylindrical flow exhibits a similar minimum flow-rate, but in practice the cone angle formed by the material is appreciably smaller than 60°.

Discrepancies appear in Figs. 3 and 4 for small glass beads, which are extremely mobile: this might be due to the effective wedge or cone angle being appreciably smaller than those reported in Table 1.

Also shown in Fig. 3 are data (Table 2) for flow of a rounded sand through troughs with half-angles of 30°, 15°, 6°, these being less than the natural wedge-angle of 56°. Two regimes of flow were found with the troughs, separated by a distinct discontinuity. At the higher slit-sizes Q was proportional to $(S - k)^{3/2}$.

Table 2. Flow through Troughs and Conical Hoppers

Material	Symbol	Mean sieve size (cm.)	Bulk density† (ρ gm./c.c)	Trough or hopper angle β deg.	Observed σ or γ	
					Low	High
		Plane flow (Fig. 3)				
Rounded sand	×, ●	c. 0·055	1·50*	30	0·89	0·99
				15	1·06	1·29
				6	1·40	1·81
		Cylindrical flow (Fig. 4)				
Radish seed (ref. 3)	R	0·25	0·73	27	0·71	
				10	1·15	
Socony beads (ref. 3)	S	0·43	0·73	27	0·73	
				10	1·38	
Potassium nitrate (ref. 4)	○	0·043	1·24	30	0·78	
				15	1·03	
	●	0·025	1·27	30	0·83	
				15	1·09	

* Assumed.
† As reported, a normal, not a minimum bulk density.

with the same k as for a flat-bottomed vessel; these points (●) follow the theoretical curve.

At the smaller slit-sizes Q still varied with $(S-k)^{3/2}$ but now k varied with trough angle and the flow-rates (×) were lower. Possibly, the 'crowdedness' of the emerging granules is greater.

Results for cylindrical flow through conical hoppers are given in Fig. 4, by recalculating data reported by other workers (Table 2). Agreement with the theory is satisfactory.

The minimum energy theory applied to radial flow from wide vessels shows that σ or γ are determined respectively by the angle of the wedge or cone formed by the material itself, or for flow through a trough or conical hopper, by the angle of the trough or hopper. For flat-bottomed vessels the difference in β for plane and for cylindrical flow is an important fact for elucidation of the observed flow-rates.

Acknowledgment is made to the British Coal Utilization Research Association for permission to publish and to Mr. J. C. Richards and Mrs. J. Tee for assistance with the experiments.

[1] Brown, R. L., and Richards, J. C., *Trans. Inst. Chem. Eng.*, **37**, 108 (1959).
[2] Brown, R. L., and Richards, J. C., *Trans. Inst. Chem. Eng.*, **38**, 243 (1960).
[3] Rausch, J. M., Ph.D. thesis, University of Princeton (1949).
[4] Deming, W. E., and Mehring, A. L., *Indust. Eng. Chem.*, **21**, 661 (1929).

COMPARATIVE ANALYSIS OF INACTIVATING EFFICIENCY OF RADIATION ON DIFFERENT ORGANISMS

By Dr. M. TERZI
Istituto di Genetica, Università, Pavia

In 1953, Epstein[1], and after him other authors, put forward an interpretation of the lethal effect (direct effect) of ionizing radiations based on the idea that the effect itself is exerted upon the nucleic acids. The dose – effect relationship was expressed as the ratio of volume of nucleic acids (calculated by other physical measurements) to 'target' volume. Their theory, however, suffered some limitations, since it gave an unsatisfactory fit when organisms other than small viruses were studied.

This interpretation can now be revised in the light of new structural data on nucleic acids, and extended to more complex organisms.

Available data are summarized in Table 1, which shows the following:

(*a*) The X-ray dose, expressed in roentgens, corresponding to $\exp^{-1}$ individuals surviving (D_0); such a dose is calculated from the logarithmic portion of the survival curve to radiations when the organisms were in a protecting medium or in a dry state. Survival is defined on the basis of infecting ability for viruses, or colony-forming ability for other organisms.

(*b*) The nucleic acid content of several organisms: for ribonucleic acid (RNA) viruses, it gives the RNA content; for all other organisms, the amount of deoxyribonucleic acid (DNA) is given: both are expressed in molecular weight units. Data were selected by choosing the most recent observations available in the literature. I have given two values when two limits are given by the same author, and also when two divergent results were published almost simultaneously. In order to reduce the number of references, I have often referred to reviews rather than to original papers.

(*c*) The lethal efficiency (e) per ion pair produced in the nucleic acids corresponding to the dose D_0.

(*d*) The mean energy (in ergs) absorbed by nucleic acids; this value is termed E and it is inversely proportional to the efficiency of inactivation.

The values for e and E are plotted in Fig. 1 in a randomized order; this procedure (suggested to me by Prof. M. Ageno) enables a non-biased evaluation of the groupings.

One can see that four different levels, expressing inactivation efficiency, correspond to four systematically distinct groups. The different degree of complexity of their genetic structure justifies their reaction to ionizing radiations.

The first level ($\bar{e} = 0{\cdot}64$) corresponds to single-stranded DNA or RNA viruses; such data are in good agreement with those obtained by Hutchinson[2] ($\bar{e} = 0{\cdot}51$) with some forty dried proteins irradiated with electrons, and with the results of Aurisicchio *et al.*[3] on the efficiency of assimilated phosphorus-32 decay of alpha phage.

At the second level one finds animal and bacterial viruses containing double-stranded DNA. In this group $\bar{e} = 0{\cdot}62 \times 10^{-1}$ in agreement with the figure calculated by Hershey *et al.*[4] for the assimilated phosphorus-32 decay of the T-even phages. The vaccinia virus (No. 2) shows an aberrant e value; some of its properties, however, such as its enzyme content[5] and the presence of many 'targets'[6], point independently to an unusual structure. It is not clear, on the other hand, why *Haemophilus influenzae* should belong to this group.

It is worth pointing out that the differences in E values between the first and second group cannot depend on the particle size, because both groups are extremely variable in this respect.

In the third group ($\bar{e} = 1{\cdot}23 \times 10^{-2}$), bacteria and haploid yeast are represented. The finding (Morse *et al.*[7]) that crossing-over in *E. coli* occurs at the four-strand stage indicates that the hereditary structure

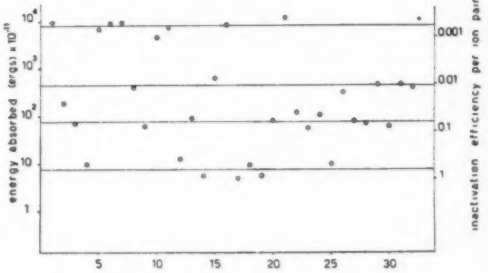


Fig. 1

Table 1

Organisms	D_0	Ref.	Nucleic acid content (mol. wt. units)	Ref.	e	E ($\times 10^{-11}$)
Tobacco mosaic virus	$1{\cdot}8 \times 10^5$	1	$2{\cdot}0 \times 10^6$	2	1·00	5·2
Tomato bushy stunt	$4{\cdot}5 \times 10^5$	3	$1{\cdot}6 \times 10^6$	4	0·51	10·2
Fowl plague	$1{\cdot}0 \times 10^5$	3	$2{\cdot}2$–$6{\cdot}0 \times 10^6$	5	1·70–0·62	3·1–8·4
Tobacco ringspot	$4{\cdot}6 \times 10^5$	3	$1{\cdot}5 \times 10^6$	6	0·54	9·7
Tobacco necrosis	$6{\cdot}7 \times 10^5$	3	$1{\cdot}5 \times 10^6$	6	0·38	13·6
Phage alpha	$2{\cdot}2 \times 10^4$	7	$3{\cdot}1 \times 10^7$	7	0·55	9·4
Phage φ *X*174 (*S* 13)	$2{\cdot}5 \times 10^5$	8	$1{\cdot}7 \times 10^6$	9	0·87	6·0
Vaccinia	$8{\cdot}0 \times 10^4$	3	$1{\cdot}8 \times 10^8$	5	$0{\cdot}26 \times 10^{-1}$	200
Shope papilloma	$4{\cdot}4 \times 10^5$	3	$1{\cdot}4 \times 10^7$ *	10	$0{\cdot}61 \times 10^{-1}$	86
Phage *BM*	$1{\cdot}9 \times 10^5$	11	$2{\cdot}5 \times 10^7$	12	$0{\cdot}81 \times 10^{-1}$	64
Phage *T1*	$8{\cdot}5 \times 10^4$	13	$5{\cdot}0 \times 10^7$	13	$0{\cdot}87 \times 10^{-1}$	60
Phage *T2* (*T4*–*T6*)	$4{\cdot}0 \times 10^4$	13	$1{\cdot}7 \times 10^8$	13	$0{\cdot}64 \times 10^{-1}$	93
Phage *T3* (*T7*)	$8{\cdot}5 \times 10^4$	13	$6{\cdot}5 \times 10^7$	13	$0{\cdot}68 \times 10^{-1}$	77
Phage *T5*	$3{\cdot}5 \times 10^4$	13	$1{\cdot}3 \times 10^8$	13	$0{\cdot}81 \times 10^{-1}$	64
Phage lambda	$1{\cdot}1 \times 10^5$	13	$7{\cdot}0 \times 10^7$	13	$0{\cdot}48 \times 10^{-1}$	108
Phage *P22*	$1{\cdot}1 \times 10^5$	13	$5{\cdot}0 \times 10^7$	13	$0{\cdot}67 \times 10^{-1}$	77
Phage *P8*	$8{\cdot}3 \times 10^4$	13	$7{\cdot}0$–$14{\cdot}0 \times 10^7$	13	$0{\cdot}64$–$0{\cdot}32 \times 10^{-1}$	82–160
Hæmophilus influenzae	$5{\cdot}0 \times 10^5$	14	$1{\cdot}2 \times 10^9$	14	$0{\cdot}62 \times 10^{-1}$	84
Bacterium aertrycke	$4{\cdot}0 \times 10^3$	3	$7{\cdot}2 \times 10^8$	15	$1{\cdot}3 \times 10^{-2}$	400
Diplococcus pneumoniae	$2{\cdot}0$–$3{\cdot}0 \times 10^4$ †	16	$1{\cdot}2 \times 10^9$	14	$1{\cdot}5$–$1{\cdot}0 \times 10^{-2}$	347–520
Micrococcus pyogenes aureus	$4{\cdot}0 \times 10^3$	17	$8{\cdot}5 \times 10^8$	15	$1{\cdot}1 \times 10^{-2}$	477
Aerobacter aerogenes	$1{\cdot}4 \times 10^3$	17	$1{\cdot}7 \times 10^{10}$	15	$1{\cdot}5 \times 10^{-2}$ ‡	337
Escherichia coli	$4{\cdot}0 \times 10^3$	18	$4{\cdot}8$–$13{\cdot}0 \times 10^9$	19–20	$1{\cdot}9$–$0{\cdot}74 \times 10^{-2}$	281–702
Saccharomyces cerevisiae (haploid)	$3{\cdot}0 \times 10^3$	21	$1{\cdot}6 \times 10^{10}$	22	$0{\cdot}77 \times 10^{-2}$	676
S. cerevisiae (diploid)	$2{\cdot}1 \times 10^4$	21	$3{\cdot}3 \times 10^{10}$	22	$0{\cdot}54 \times 10^{-3}$	9,670
(triploid)	$1{\cdot}3$–$1{\cdot}8 \times 10^4$	21	$4{\cdot}5 \times 10^{10}$	22	$0{\cdot}65$–$0{\cdot}46 \times 10^{-3}$	8,000–11,200
(tetraploid)	$1{\cdot}3 \times 10^4$	21	$6{\cdot}8 \times 10^{10}$	22	$0{\cdot}42 \times 10^{-3}$	12,400
(pentaploid)	$7{\cdot}7 \times 10^3$	21	$8{\cdot}0 \times 10^{10}$	22	$0{\cdot}60 \times 10^{-3}$ §	8,680
(hexaploid)	$7{\cdot}4 \times 10^3$	21	$9{\cdot}6 \times 10^{10}$	22	$0{\cdot}52 \times 10^{-3}$ §	9,980
Guinea pig	$1{\cdot}0 \times 10^2$	23	$5{\cdot}0 \times 10^{12}$	24	$0{\cdot}74 \times 10^{-3}$	7,020
Chick	$3{\cdot}0$–$4{\cdot}0 \times 10^2$	25	$1{\cdot}5 \times 10^{12}$	24	$0{\cdot}81$–$0{\cdot}62 \times 10^{-3}$	6,400–8,420
Man	$5{\cdot}0$–$6{\cdot}0 \times 10^1$	26	$6{\cdot}2 \times 10^{12}$	24	$1{\cdot}20$–$1{\cdot}00 \times 10^{-3}$	4,350–5,200

*, the value calculated in 1942 by Taylor *et al.* was 4×10^6. †, γ-rays. ‡, assuming 4 per cent dry weight its DNA content. §, assuming that the same relation between ploidy and amount of DNA found up to tetraploid holds up to hexaploid.

[1] Wohlhieter, J. A., Buzzell, A., and Lauffer, M. A., *Biochim. Biophys. Acta*, **43**, 163 (1960). [2] Friesen, B. S., and Sinsheimer, R. L., *J. Mol. Biol.*, **1**, 321 (1959). [3] Lea, D. E., *Action of Radiations on Living Cells* (Camb. Univ. Press, second edit., 1956). [4] Schachman, H. K., and Williams, R. C., on *Viruses*, edit. by Burnet, F. M., and Stanley, W. M., **1**, 316 (Acad. Press, New York, 1959). [5] Schäfer, W., on *Viruses*, edit. by Burnet, F. M., and Stanley, W. M., **1**, 475 (Acad. Press, New York, 1959). [6] *The Nucleic Acids*, **3**, 217; edit. by Chargaff, E., and Davidson, J. (Acad. Press, 1960). [7] Aurisicchio, S., Celano, A., Coppo, A., Donini, P., and Graziosi, F., *Supp. Nuovo Cim.* (in the press). [8] Tessman, I., Tessman, E. S., and Stent, G. S., *Virology*, **4**, 209 (1957). [9] Sinsheimer, R. L., *J. Mol. Biol.*, **1**, 43 (1959). [10] Watson, J. D., and Littlefield, J. M., *J. Mol. Biol.*, **2**, 161 (1960). [11] Castagnoli, C., Donini, P., and Graziosi, F., *Giorn. Microbiol.*, **1**, 86 (1959). [12] Aurisicchio, S., Cortini, G., Emma, V., and Graziosi, F., *Int. J. Rad. Biol.*, **1**, 86 (1959). [13] Stent, G. S., *Adv. Virus Res.*, **5**, 98, edit. by Smith, K. M., and Lauffer, M. A. (Acad. Press, 1958). [14] Stuy, J. H., *J. Bact.*, **79**, 707 (1960). [15] Bacterial volume taken from Bergey's *Manual of Determinative Bacteriology*, edit. Bread, S. B., Murray, E. G. D., and Hitchens, A. P. (Baltimore, William and Wilkins, 1948). DNA content taken from Davidson, J. N., *Biochemistry of Nucleic Acids* (Methuen, 1950). [16] Drew, R. M., *Rad. Res.*, **3**, 116 (1955). [17] Fram, H., Proctor, B. E., and Dunn, C. G., *J. Bact.*, **60**, 263 (1950). [18] Pollard, E., *Amer. Nat.*, **94**, 71 (1960). [19] Gillies, N. E., and Alper, T., *Biochim. Biophys. Acta*, **43**, 182 (1960). [20] Harold, F. M., and Ziporin, Z. Z., *Biochem. Biophys. Acta*, **28**, 482 (1958). [21] Mortimer, R. K., *Rad. Res.*, **9**, 312 (1958). [22] Ogur, M., Minckler, S., Lindegren, G., and Lindegren, C. G., *Arch. Biochem. Biophys.*, **40**, 175 (1952). [23] Marin, G., and Colombo, B., *Atti Congr. A.G.I.* (Roma, in the press). [24] Leslie, I., in the *Nucleic Acids*, **2**, 1, edit. by Chargaff, E., and Davidson, J. N. (Acad. Press, 1955). [25] Rubin, H., and Temin, H. M., *Virology*, **7**, 75 (1959). [26] Puck, T. T., *J. Cell. Comp. Phys.*, **52**, Supp. 1, 287 (1000). [27] Ginoza, W., and Norman, A., *Nature*, **179**, 520 (1957). [28] Goodgal, S. H., Herriott, R. M., *Chemical Bases of Heredity*, edit. by McElroy, W. D. and Glass, B., 336 (Johns Hopkins Press, Baltimore, 1957). [29] Stuy, J. H., *Rad. Res.* (in the press). [30] Lerman, L. S., and Tolmach, L. J., *Biochim. Biophys. Acta*, **33**, 371 (1959). [31] Doty, P., quoted by Shooter, K. V., in *Progr. in Biophys.*, **8**, 310 (1957). [32] de Filippes, F. M., and Guild, W. R., *Rad. Res.*, **11**, 38 (1959).

Table 2

Organisms	D_0	Ref.*	Nucleic acid content (mol. wt. units)	Ref.*	e	E ($\times 10^{-11}$)
Tobacco mosaic virus	$1{\cdot}8 \times 10^5$	1	$2{\cdot}0 \times 10^6$	2	1·0	5·2
RNA	$1{\cdot}3 \times 10^5$	1–27	$2{\cdot}0 \times 10^6$	2	0·9	5·8
Hæmophilus influenzae	$5{\cdot}0 \times 10^5$	14	$1{\cdot}2 \times 10^9$	14	0·062	84
Transforming principle DNA	$1{\cdot}7 \times 10^5$	14	$1{\cdot}5$–3×10^7	28–29	0·100	52
Diplococcus pneumoniae	$2{\cdot}3 \times 10^4$	16	$1{\cdot}2 \times 10^9$	14	0·012	435
Transforming-principle slow	$2{\cdot}3 \times 10^4$	30	$7{\cdot}7 \times 10^6$	31	0·028	18
Transforming-principle fast	$5{\cdot}0 \times 10^5$	32	$7{\cdot}7 \times 10^6$	31	0·100	52

* For references, see footnotes Table 1.

of bacteria is closer to that of yeast (a conventional halpoid organism) than to that of bacteriophages.

It may be mentioned that isolated DNA or RNA of different organisms (tobacco mosaic virus, *Haemophilis influenzae*, *Diplococcus pneumoniae*) show the same e value as the organism from which they are derived (see Table 2). In the case of *Diplococcus pneumoniae*, the transforming principle shows a two-slope survival curve. The value of E calculated from the slowly inactivated part shows no significant departure from E for the whole cell, while the E calculated from the fast inactivated portion of the curve is close to the E of the second group. But it is not known whether the molecular weights of the two fractions—if there are actually two fractions—are equal or not. No conclusion can therefore be reached here.

Cells from vertebrates, including man, and from yeast with ploidy greater than 1, can be placed together in the fourth group ($\bar{e} = 0{\cdot}69 \times 10^{-3}$).

The fact that the inactivating efficiency for organisms of ploidy greater than 1 is lower than that for haploid cells is shown qualitatively by the different slopes of the exponential portion of the survival curves of haploid and diploid yeast cells[8]. The different efficiencies have been interpreted as due to different killing mechanisms. In haploid cells, recessive lethals (probably point mutations and/or small

deficiencies) account for a rather high proportion of total lethality, whereas in polyploid organisms the most efficient type of lethals is the dominant factor (probably chromosomal aberrations or breakages).

It is difficult to offer a physical interpretation of these results. From a biological point of view, it can be said that there are discrete levels of structural organization corresponding to well-defined degrees of radiosensitivity, which seems to decrease with increasing structural complexity from single-stranded DNA to the chromosome of haploid organisms. The further decrease of radiosensitivity from haploid to di- and poly-ploid could be due, however, to a change in the phenogenetic pattern of lethality rather than to increased complexity.

It would be very useful to collect new data and compare them with other parameters related to radiosensitivity. I was limited to the use of those unicellular organisms and those cells behaving like colony-forming organisms, because colony formation appears to be a uniform and reliable way of comparing the efficiency of ionizing radiation. From a physical point of view, it can be suggested that the inactivation of more complicated structures needs perhaps larger ionization clusters, which are obviously rarer. It would also be interesting to compare the above data with others on the mutation frequency in cistrons of known length, to see if gene mutation can also show different degrees of radiosensitivity in organisms of different complexity.

I am indebted to Prof. F. Graziosi, of the University of Rome, for very valuable discussion during the preparation of this communication. The work was supported by a grant from C.N.E.N., Rome.

[1] Epstein, H. T., *Nature*, **171**, 394 (1953).
[2] Hutchinson, F., *Amer. Nat.*, **94**, 59 (1960).
[3] Aurisicchio, S., Celano, A., Coppo, A., Donini, P., and Graziosi, F., Supp. *Nuovo Cim.* (in the press).
[4] Stent, G. S., *Adv. Virus Res.*, **5**, 98, edit. Smith, K. M., and Lauffer, M. A. (Acad. Press, 1958).
[5] Schäfer, W., on *Viruses*, edit. by Burnet, F. M., and Stanley, W. M., **1**, 475 (New York, Acad. Press, 1959).
[6] Lea, D. E., *Action of Radiations on Living Cells*, second edit. (Camb. Univ. Press, 1956).
[7] Morse, M. L., Lederberg, E. M., and Lederberg, J., *Genetics*, **41**, 758 (1956).
[8] Magni, G. E., Supp. to *Nuovo Cim.* (in the press).

FORMATION AND UTILIZATION OF POLY-β-HYDROXYBUTYRIC ACID BY KNALLGAS BACTERIA (*HYDROGENOMONAS*)

By Prof. H. G. SCHLEGEL, G. GOTTSCHALK and R. VON BARTHA
Institut für Mikrobiologie der Universität, Göttingen

THE conclusion that hydrogen-oxidizing bacteria of the *Hydrogenomonas* type are rich in storage material and are able to synthesize it from carbon dioxide and molecular hydrogen has been drawn from three types of experiments: (*a*) the intensity of endogenous respiration remains nearly constant for relatively long periods of aerobic incubation; (*b*) when chemolithotrophically growing cells have reached the stationary growth phase, turbidity continues to increase while the total cell count remains constant; (*c*) washed cells suspended in phosphate buffer oxidize hydrogen in the presence of carbon dioxide three times as fast as they do in its absence. In these conditions, carbon dioxide is assimilated in substrate amounts and incorporated into a cell fraction which amounted to 65 per cent of the dry weight of cells. The solubility in chloroform, the insolubility in ethyl-ether and petroleum ether, and the resistance to potassium hypochlorite solution indicated that the storage material was poly-β-hydroxybutyric acid. This polymeric material is accumulated in the cells when, in the absence of nitrogen or phosphorus, protein synthesis and growth are inhibited, or when the cells have reached the stationary phase of growth.

For identification of the storage material three strains of *Hydrogenomonas* (*H*1, *H*16 and *H*20) were used which had been enriched under chemolithotrophic conditions and isolated independently from each other. The strains differed from one another in several physiological characteristics. They were grown in submerged culture[1]. At the end of the exponential growth phase, the cells were collected, washed and suspended in phosphate buffer. The suspension was stirred (650 r.p.m.) in an atmosphere of hydrogen (60 per cent), oxygen (30 per cent), and carbon dioxide (10 per cent) at 28° C. for 30 hr. In other experiments the nitrogen source in the growth medium was restricted to 300 mgm. ammonium chloride/litre. After having reached a density of 0·75 mgm. dry weight/ml., the cells stopped growing; further increases in dry weight and turbidity were caused by the synthesis of poly-β-hydroxybutyric acid (Table 1).

Table 1. Increases in Dry Weight and in Poly-β-hydroxybutyric Acid Content in *Hydrogenomonas H*20 during Chemolithotrophic Growth following Depletion of the Nitrogen Source

Incubation time (hr.)	Cell dry weight (mgm./ml.)	Poly-β-hydroxybutyric acid (mgm./ml.)
42	0·75	0·234
111	1·55	1·012
Increase	0·80	0·778

Cells were grown in a mineral medium containing 300 mgm. ammonium chloride/litre in an atmosphere of 60 per cent hydrogen + 30 per cent oxygen + 10 per cent carbon dioxide, *p*H 7·0, 28° C. Cell protein increased up to the thirty-ninth hour and then remained constant.

The accumulation of poly-β-hydroxybutyric acid during the fixation of carbon dioxide in the absence of a source of nitrogen can be demonstrated in a series of micrographs of thin sections (Fig. 1, *a*–*c*.) In log-phase cells no structures other than nuclear material can be detected. After a 12-hr. period of carbon dioxide fixation, several round and oval areas become visible which show a distinct boundary and appear empty. These areas undoubtedly are inclusions of poly-β-hydroxybutyric acid. After a 48-hr. period of carbon dioxide fixation, the whole cell is filled with a few large inclusions of poly-β-hydroxybutyric acid which displace the chromatin bodies and the cytoplasm into a minimum possible area at the periphery and the interstitial areas between the inclusions. Since the density of these areas is even less than the

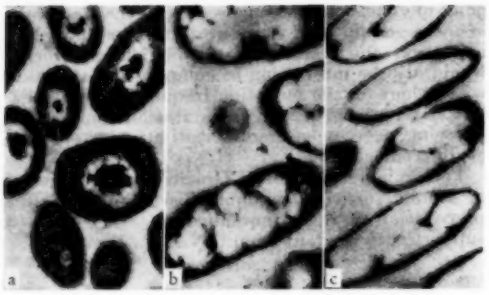


Fig. 1. Electron-micrographs of thin sections of *Hydrogenomonas H16* showing the formation of inclusions of poly-β-hydroxybutyric acid during incubation in phosphate buffer under an atmosphere o 70 per cent hydrogen + 20 per cent oxygen + 10 per cent carbon dioxide for 0 hr. (*a*, log-phase cells), 12 hr. (*b*) and 48 hr. (*c*) Fixation for 2 hr. at 3° C. with 0·5 per cent osmic acid at *p*H 8·0 (veronal acetate buffer); embedded in methacrylate. Electron-microscopical preparations and studies were carried out by Mr. K. Kran. (× 21,500)

density of the surrounding methacrylate of the section, it is concluded that the poly-β-hydroxybutyric acid dilutes the embedding medium.

In order to isolate the storage material, the washed cells were treated with potassium hypochlorite solution (Williamson and Wilkinson's method[2]). The residue, washed with water, ethanol and ethyl-ether, was almost completely dissolved in hot chloroform and after filtration could be re-precipitated with ether. Using this method at a temperature of 0° C., 0·4 vol. of ether will precipitate 7·4 per cent of the storage material, while 0·52 vol. precipitates 33·0 per cent and 1·2 vol. 100 per cent. The melting points and analytical determinations of the purified storage materials derived from *H1*, *H16* and *H20* are shown in Table 2. Furthermore, poly-β-hydroxybutyric acid preparations were analysed which had been isolated from *Bacillus megaterium* and *Azotobacter chroococcum*, in both of which poly-β-hydroxybutyric acid had previously been found[3,4]. The different melting-point intervals of the preparations indicated different degrees of polymerization.

Table 2. Melting Points and Analytical Results for Poly-β-hydroxybutyric Acids isolated from Different Bacteria

Organism	Melting point	C (per cent)*	H (per cent)*
Azotobacter chroococcum	150–155	55·4	7·0
Bacillus megaterium	168–173	55·6	6·9
Hydrogenomonas H1	162–166	55·6	7·1
" *H16*	158–162	55·4	7·0
" *H20*	167–172	55·6	7·4

* The theoretical values calculated for $(C_4H_6O_2)_n$ are: C = 55·8 per cent; H = 7·0 per cent.

The infra-red spectra of all five substances examined by us were identical. Dry distillation of poly-β-hydroxybutyric acid gave crotonic acid with a yield of 70 per cent. After recrystallization from water, this crotonic acid melted at 70° C. Crotonic acid amide prepared from this acid had a melting point of 160° C. These two derivatives, prepared from extracted poly-β-hydroxybutyric acid, were found to be identical with pure crotonic acid and the crotonic acid amide prepared from it. No depression of the melting points was observed on mixing the substances obtained from extracted poly-β-hydroxybutyric acid with the chemically pure compounds. Free β-hydroxybutyric acid could be prepared by means of alkaline hydrolysis of poly-β-hydroxybutyric acid and ether extraction of the acidified hydrolysate; this was verified by determination of the optical activity and using paper chromatography and reference substances.

In addition to the method for the preparation of poly-β-hydroxybutyric acid described by Williamson and Wilkinson[2], we have had good results with chloroform extraction of lyophilized cells. A rather pure product is prepared with quantitative yield when the dry powder is extracted with chloroform at room temperature, and the poly-β-hydroxybutyric acid precipitated with ether. Methylene chloride is as efficient as chloroform in extracting it.

The efficiency with which poly-β-hydroxybutyric acid is synthesized from carbon dioxide by oxidation of hydrogen was determined by a manometric method in a Warburg apparatus using $NaH^{14}CO_3$. The stoichiometric relationship of the gas mixture consumed to the carbon dioxide taken up was found to be 8–8·5 under the conditions of synthesis of storage material. It does not vary significantly from the corresponding relationship under growth conditions and agrees with the equation:

$$25H_2 + 8O_2 + 4CO_2 \rightarrow (C_4H_6O_2) + 22H_2O$$

During organotrophic metabolism poly-β-hydroxybutyric acid is also stored by *Hydrogenomonas* in considerable quantities (Fig. 2). With β-hydroxybutyric acid or crotonic acid serving as substrate, the speed with which the storage material is synthesized is even greater than in autotrophic conditions. When the cells are incubated with these substrates, it makes practically no difference whether they are in contact with air or with a hydrogen–oxygen mixture. Since

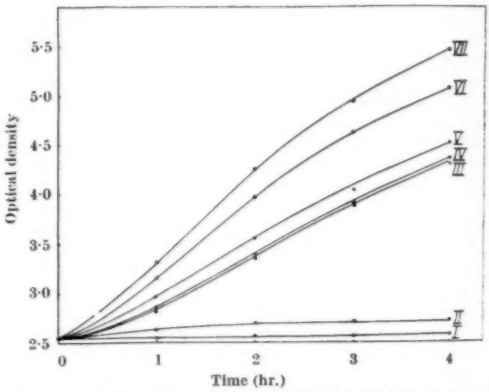


Fig. 2. Synthesis of poly-β-hydrobutyric acid by *Hydrogenomonas H16* in nitrogen-free mineral medium with different sources of energy and carbon

I. Under air without carbon source	0·00	Increase in poly-β-hydroxybutyric acid (in mgm./mgm. cell protein) in the course of 4 hr. incubation
II. Under nitrogen with β-hydroxybutyric acid	—	
III. Under air with crotonic acid	0·41	
IV. Under hydrogen + oxygen with crotonic acid	0·42	
V. Under hydrogen + oxygen + carbon dioxide	0·47	
VI. Under hydrogen + oxygen with β-hydroxybutyric acid	0·54	
VII. Under air with β-hydroxybutyric acid	0·66	

20 ml. suspension (1 mgm. cell protein/ml.) in 200 ml. Erlenmeyer flasks were shaken at 30° C. under the conditions indicated (with 1 m*M* β-hydroxybutyric acid or crotonic acid per flask); measurement of turbidity at 436 mμ; determination of poly-β-hydroxybutyric acid gravimetrically after lyophilization of 10 ml. suspension, chloroform extraction and following precipitation with ether

only small amounts of energy seem to be necessary for the polymerization of the monomers taken up by the cell, these may easily be produced by substrate oxidation, with the result that the efficiency of the synthesis cannot be increased by simultaneous hydrogen oxidation. Other organic substrates have not yet been examined.

Cells containing little storage material (less than 10 per cent poly-β-hydroxybutyric acid) and those containing a great deal (more than 50 per cent) differ considerably in their endogenous respiration (Table 3). The former were collected prior to the end of the exponential growth phase, the latter 20 hr. after depletion of the nitrogen source. Oxygen uptake was determined manometrically with cell suspensions in phosphate buffer with and without addition of a nitrogen source (ammonium chloride).

Table 3. Endogenous Respiration of Cells containing Small and Large Amounts of Poly-β-hydroxybutyric Acid (μl. oxygen/mgm. protein/hr.)

Elapsed time (hr.)	PHBA-poor cells		PHBA-rich cells	
	$-NH_4Cl$	$+NH_4Cl$	$-NH_4Cl$	$+NH_4Cl$
2	12·4	16·0	22·7	37·2
5	4·5	4·7	16·2	30·8
8	3·0	3·2	14·6	25·9

Warburg vessels with 2·5 ml. suspension of *Hydrogenomonas H*20 in 0·06 *M* phosphate buffer at *p*H 7·0; 113·2 μ*M* ammonium chloride/vessel; 0·2 ml. 20 per cent potassium hydroxide in central well; atmosphere, air; 30° C.

The endogenous respiration of rich cells is not only greater than that of cells containing little poly-β-hydroxybutyric acid, but is also greatly increased by the introduction of a nitrogen source. This boost in uptake of oxygen over and above endogenous respiration, which is brought about by the ammonium ion and which is not observed with cells poor in poly-β-hydroxybutyric acid, seems to indicate that the accumulated poly-β-hydroxybutyric acid is re-circulated into the metabolism and used for protein synthesis.

This hypothesis was verified by experiments in which the increase and decrease of poly-β-hydroxybutyric acid and cellular nitrogen were measured during endogenous respiration and during hydrogen oxidation, without carbon dioxide, of cells rich in poly-β-hydroxybutyric acid with and without a nitrogen source (Table 4).

Table 4. Re-utilization of Poly-β-hydroxybutyric Acid for Protein Synthesis by *Hydrogenomonas H*16

Experimental conditions		Cell content		Difference after 12 hr.	
N-source	Atmosphere	Total N (mgm.)	PHBA (mgm.)	Total N (mgm.)	PHBA (mgm.)
Initial suspension		0·358	2·50	—	—
None	Air	0·369	2·30	+0·011	−0·2
Ammon. chloride	Air	0·546	0·67	+0·188	−1·83
None	70 per cent H_2 + 30 per cent O_2	0·367	2·47	+0·009	−0·03
Ammon. chloride	70 per cent H_2 + 30 per cent O_2	0·622	0·61	+0·264	−1·89

Suspension of chemolithotrophically grown cells (50 per cent poly-β-hydroxybutyric acid) in 0·067 *M* phosphate buffer at *p*H 7·0; 75 μ*M* ammonium chloride/ml.; 28° C.; atmosphere, air or carbon dioxide-free hydrogen–oxygen mixture (70 per cent hydrogen + 30 per cent oxygen; carbon dioxide absorbed by 20 per cent potassium hydroxide); incubation time 12 hr. (with stirring); Kjeldahl method used to determine cellular nitrogen; poly-β-hydroxybutyric acid determined gravimetrically from lyophilized material after extraction with chloroform and precipitation with ether.

Under air, only a small percentage of the poly-β-hydroxybutyric acid is consumed by endogenous respiration. In the presence of ammonium chloride, the cellular nitrogen increases considerably at the expense of poly-β-hydroxybutyric acid. In an atmosphere of carbon dioxide-free hydrogen–oxygen mixture these effects are even more pronounced: while the poly-β-hydroxybutyric acid decreases, the cellular nitrogen accumulates to a considerable extent. Therefore we must assume that we are dealing with poly-β-hydroxybutyric acid as a genuine storage material, which in the absence of a carbon source can support metabolism, and can be used for protein synthesis in the presence of a nitrogen source. The efficiency of this synthesis is greater during hydrogen oxidation than during endogenous respiration.

The three strains of *Hydrogenomonas* examined up to the present time synthesize poly-β-hydroxybutyric acid chemolithotrophically as well as organotrophically and are able to re-utilize the storage material in their metabolism.

We acknowledge the support of the Deutsche Forschungsgemeinschaft in this work.

[1] Schlegel, H. G., Kaltwasser, H., and Gottschalk, G., *Arch. Mikrobiol.*, **38**, 209 (1961).
[2] Williamson, D. H., and Wilkinson, J. F., *J. Gen. Microbiol.*, **19**, 198 (1958).
[3] Lemoigne, M., *Bull. Soc. Chim. Biol.*, **8**, 770 (1926).
[4] Lemoigne, M., and Girard, H., *C.R. Acad. Sci., Paris*, **217**, 557 (1943).

APPARENT CYTOPLASMIC INHERITANCE OF TYROSINASE COMPETENCE IN *STREPTOMYCES SCABIES*

By Prof. K. F. GREGORY and WEI-JEN SHYU
Department of Microbiology, Ontario Agricultural College, Guelph

Streptomyces scabies, an etiological agent of potato scab, like other members of its genus, resembles the true bacteria in size and many physiological characteristics, but has a mycelial growth habit. The formation of nutritionally balanced heterokaryotic growth between pairs of nutritionally deficient strains has been demonstrated in several species of *Streptomyces*[1–3]. Infrequent genetic recombination, presumably preceded by nuclear fusion, occurs in some of these species, but the great majority of the spores formed by the heterokaryons have the nutritional requirements of one or the other of the two parental strains. Since the two parental-type nuclei share the same cytoplasm within the heterokaryon, any cytoplasmically inherited characteristic should be shared by uni-nucleate spores bearing both types of nuclei. This phenomenon appears to occur with the determinant for tyrosinase production in *Streptomyces scabies*.

Strains of *S. scabies* isolated from potato scab lesions are normally capable of forming a dark brown pigment (presumably melanin) in tyrosine-containing

medium in contact with air. Tyrosinase-deficient mutants arise at a relatively high frequency, however, comprising about 0·2 per cent of the total colonies derived from non-irradiated spores and up to 5 per cent of the colonies developing from X-ray treated spores[4]. The tyrosinase-deficient state, by contrast, was found to be highly stable, and no back mutation to tyrosinase competence has been detected, following numerous platings of tyrosinase-deficient mutants during a 5-year period.

Strains of *S. scabies* have been observed cytologically to undergo frequent hyphal fusions[5]. Colonies, concluded to be nutritionally balanced heterokaryons, formed readily on minimal medium, when some pairs of mutants, each bearing two complementary nutritional deficiencies, were plated together. The great majority of the spores derived from these heterokaryons had the nutritional requirements of one or the other of the two parents, but usually in unequal proportions. Thus, in a cross between *S. scabies* strains *A*26–34 (nicotinic acid⁻, biotin⁻, tyrosinase⁻, streptomycin sensitive) and *A*26–37 (arginine⁻, methionine⁻, tyrosinase⁺, streptomycin resistant) the spores formed by the heterokaryon reproduced the two patterns of parental nutritional deficiencies in a ratio of about 50 : 1, respectively, although the ratio varied somewhat from experiment to experiment (Table 1). When platings were made from heterokaryotic colonies on to minimal medium, most of the few colonies which developed were found to be heterokaryons and may have developed from fragments of mycelium or might have arisen *de novo* from the two parental types present. When platings were made from heterokaryons on to nutritionally selective media, essentially all the colonies of both parental nutritional types gave a strong tyrosinase reaction, the incidence of tyrosinase-deficient colonies being no greater than that observed with normal tyrosinase-producing cultures.

Table 1. Cytoplasmic Inheritance of Tyrosinase Competence via Heterokaryon Formation

Medium	Counts per ml. of spore suspensions prepared from single colonies of:		
	*A*26–34	*A*26–37	Heterokaryon of *A*26–34 × *A*26–37
Minimal	0	0	$8·8 \times 10^2$
Minimal + nicotinic acid + biotin	$4·1 \times 10^8$	0	$3·7 \times 10^6$
Minimal + arginine + methionine	0	$2·2 \times 10^6$	$8·1 \times 10^4$
Complete + streptomycin	0	$2·0 \times 10^6$	$6·2 \times 10^4$
Per cent tyrosinase-producing colonies	0	> 99	> 99

The reverse cross yielded similar results. Thus when strain *A*26–54 (nicotinic acid⁻, biotin⁻, tyrosinase⁺, streptomycin resistant) was plated on minimal medium together with strain *A*26–53 (arginine⁻, methionine⁻, tyrosinase⁻, streptomycin sensitive) and the resulting heterokaryotic colonies plated on nutritionally differential media, once again nearly all the colonies of both parental nutritional types were tyrosinase⁺ (Fig. 1). The tyrosinase-producing abilities of colonies carrying the nutritional requirements of the formerly tyrosinase-deficient parents were shown to be perpetuated throughout at least six consecutive transfers on complete medium.

Many of the colonies developing on medium supplemented with nicotinic acid and biotin, from both crosses, required only nicotinic acid for growth. This acquisition of independence of biotin was considered to result from the relatively high frequency of back mutation to biotin⁺ ($1·6 \times 10^{-4}$ per parental spore plated) coupled with the fact that these back mutants made a greater amount of residual growth on the minimal medium than did the diauxotroph. There was thus an increased probability of heterokaryon formation with these biotin⁺ back mutants. A selection of tyrosinase⁺ back mutants during heterokaryon formation appears to have been highly unlikely since this back mutation has never been detected. Furthermore, the tyrosinase⁺ characteristic gave this culture no apparent selective advantage on the media employed.

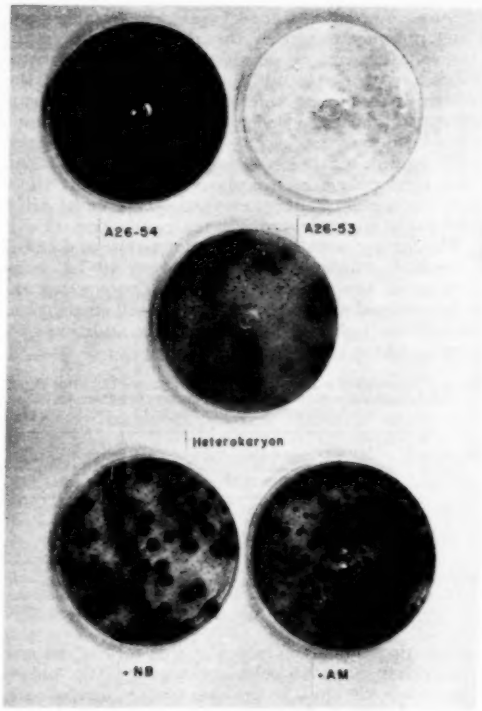


Fig. 1. Appearance of the under side of colonies of parental, heterokaryon and recovered parental nutritional types on tyrosine-containing media. Top: parental strains *A*26–54 (nicotinic acid⁻, biotin⁻, tyrosinase⁺, streptomycin resistant) and *A*26–53 (arginine⁻, methionine⁻, tyrosinase⁻, streptomycin sensitive) on complete medium. Centre: heterokaryons from the above strains on minimal medium. Bottom: colonies developing, from spores formed by heterokaryon, on minimal medium supplemented with nicotinic acid + biotin (+ *NB*) and arginine + methionine (+ *AM*)

Genetic recombination of the determinants for nutritional deficiencies and streptomycin resistance was detected by plating spores from heterokaryons on minimal medium containing various combinations of nutritional supplements and streptomycin. Excluding the nicotinic acid⁻ biotin⁺ streptomycin-sensitive class, which was not considered to arise by recombination, recombinants comprised only a small proportion (< 0·1 per cent) of the total spores formed by the heterokaryons. On the assumption that these characteristics were inherited as nuclear genes, it was apparent that the incidence of nuclear genetic recombination in these strains was not sufficient to account for the abundant coupling of tyrosinase-

producing capability with the nutritional deficiencies of the formerly tyrosinase-deficient strains.

A selective overgrowth of the heterokaryon with recombinant nuclei containing the tyrosinase$^+$ determinant was improbable in view of the short interval available for such overgrowth (development of one colony), lack of a demonstrable selective growth advantage of this characteristic in the test conditions, and the clear demonstration in *Neurospora* that even nuclei bearing genes with a strong selective advantage did not increase in proportion during the growth of a heterokaryon[6]. Even if the large number of biotin$^+$ colonies produced by the heterokaryons had resulted from the formation, by recombination, of nuclei bearing this characteristic, followed by a selective increase in the proportion of this type of nucleus within the heterokaryon, the high incidence of tyrosinase$^+$ colonies obtained clearly would not have resulted from a close linkage of this determinant to the biotin locus, since most of the biotin$^+$ colonies from *A*26–53 × *A*26–54 heterokaryons would then have been deficient in tyrosinase.

The tyrosinase$^+$ colonies obtained from the heterokaryons were shown not to have been derived from multinucleate or diploid spores, since spores of these cultures showed an exponential rate of killing by X-rays, usually characteristic of a haploid, uninucleate condition, and the other genetic markers could not be recovered afterwards from these cultures. The culture from which these genetically marked strains were derived appeared cytologically to have a single nucleus[5].

Attempts to obtain infective transfer of tyrosinase-producing ability from *A*26–37 to *A*26–34 were unsuccessful. Culturing *A*26–34 in both liquid and solid media containing 10 per cent culture filtrate or a membrane-filtered extract of sonically disrupted mycelium of *A*26–37 did not result in a single tyrosinase-producing colony. The quantitative transfer of tyrosinase-producing ability from a tyrosinase-producing strain of *S. scabies* to a tyrosinase-deficient strain did not, therefore, appear to be mediated by a free virus like the carbon dioxide sensitivity factor in *Drosophila*[7], toxigenicity in *Corynebacterium diphtheriae*[8] or some antigenic changes in *Salmonella* spp.[9].

The phenomenon described here appears to be explained best by postulating the presence of auto-reproducing constituents in the cytoplasm, which control the production of tyrosinase in this species. Strong evidence exists for cytoplasmic inheritance of several characteristics in diverse organisms[9]. Horowitz and Fling[10], however, specifically ruled out cytoplasmic inheritance of tyrosinase-producing ability in *Neurospora crassa* with data diametrically opposed to that presented here for *S. scabies*.

The support of this work by a grant from the National Research Council of Canada is gratefully acknowledged.

[1] Bradley, S. G., Anderson, D. L., and Jones, L. A., *Ann. N.Y. Acad. Sci.*, **81**, 811 (1959).
[2] Braendle, D. H., and Szybalski, W., *Ann. N.Y. Acad. Sci.*, **81**, 824 (1959).
[3] Saito, H., and Ikeda, Y., *Ann. N.Y. Acad. Sci.*, **81**, 862 (1959).
[4] Gregory, K. F., and Vaisey, E. B., *Canad. J. Microbiol.*, **2**, 65 (1956).
[5] Gregory, K. F., *Canad. J. Microbiol.*, **2**, 649 (1956).
[6] Pittenger, T. H., and Atwood, K. C., *Genetics*, **41**, 227 (1956).
[7] L'Heritier, P., *Advances in Virus Res.*, **5**, 195 (1958).
[8] Barksdale, L., *Bact. Rev.*, **23**, 202 (1959).
[9] Catcheside, D. G., *Nature*, **184**, 1012 (1959).
[10] Horowitz, N. H., and Fling, M., *Proc. U.S. Nat. Acad. Sci.*, **42**, 498 (1956).

COMPARISON OF PYRIDINE NUCLEOTIDES IN THE LIVER AND IN AN ASCITIC HEPATOMA

By M. WINTZERITH N. KLEIN, L. MANDEL and Prof. P. MANDEL

Centre de Recherches Nucléaires, Applications Biologiques, Strasbourg-Cronenbourg, and Institut de Chimie Biologique, Faculté de Médecine, Strasbourg

THE work of von Euler[1] showed that there was no difference in the diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) contents of the liver, muscle and Jensen's sarcoma in the same animal. Since then, numerous data have accumulated showing that in tumours[2–5], in tumoural mitochondria[6] and in organs in a pre-cancerous state[7] the DPN content is lower than in normal tissues. In animals with a tumour such as sarcoma 37, a lymphoma or a hepatoma, a decrease in DPN of certain cells has been noted[8,9]. However, in most cases, the comparison between normal and neoplasmic tissues concerns cells the morphology of which differs widely. Furthermore, different types of cells contain varying amounts of DPN and TPN whether in normal or neoplasmic tissues.

These reasons prompted us to compare the DPN and TPN contents in normal liver, in the Zajdela hepatoma and in the liver of animals with this tumour. Here we are concerned with homologous types of tissues and with a tumour which has not lost as much of the character of the original tissue as sarcomas or Ehrlich ascitic cells which have been transplanted many times. The hepatoma of Zajdela which we utilize maintains the trabecular structure of liver parenchyma. This is important because hepatomas can originate in various liver cells: biliar-epithelium, parenchymatous cells, reticulo-endothelial cells, and a comparison between the liver and tumour will be valid only if the latter is also of a parenchymatous nature. Pitot and Potter[10] have emphasized recently the different biochemical characters of distinct morphological types of hepatoma. In our comparison of normal and tumorous tissues we have utilized various bases of references besides the wet weight normally used. In particular, we thought it interesting to compare values related to the deoxyribonucleic acid (DNA) content, thus obtaining an indication at the cell-level.

In our comparative experiments on the pyridine nucleotides of liver and hepatoma we used twenty standardized rats, eight being for estimations on the liver and twelve for the tumours. For investigating the effects of nicotinamide we used eighteen rats, ten of which had a tumour. Reduced diphosphopyridine nucleotide (DPNH), DPN and the total TPN +

reduced triphosphopyridine nucleotide (TPNH) were determined by the enzymic method described by Holzer *et al.*[11]. After fractionation according to Schmidt and Thannhauser[12], DNA was determined by the estimation of deoxyribose by the method of Dische[13] as modified by Burton[14]. Our experimental results are recorded in Figs. 1–3.

Fig. 1 shows that the presence of an ascitic hepatoma alters only slightly the DPN and TPN + reduced TPN contents of the liver. Although the values for animals with a tumour vary within narrower limits than in normal subjects and the average results are slightly higher in the controls, the differences are not significant.

Fig. 2 shows the DPN, reduced DPN and TPN contents of liver and ascitic cells. Bearing in mind the difficulty of accurate assessment of the wet weight of ascitic cells, we related our results to accurately determinable quantities, that is, dry weight, proteins and DNA. Our results demonstrate that the values of DPN related to dry weight are higher for the liver than for ascitic cells. The same applies for reduced DPN and the triphosphopyridine nucleotides as a whole. Even though the standard deviation for the results is comparatively high the difference is statistically valid. The average values for the DPN, reduced DPN and TPN + reduced TPN contents of tumorous tissues were, respectively, 61 per cent, 77 per cent and 55·5 per cent of the values for normal liver. The percentages are of the same order, if the results are related to proteins. The differences between normal and ascitic cells are more important and highly significant if the values are related to DNA. Thus, the average values for the DPN, reduced DPN and TPN + reduced TPN of ascitic cells are only 14·4 per cent, 17·6 and 12 per cent, respectively, of the values for normal tissues.

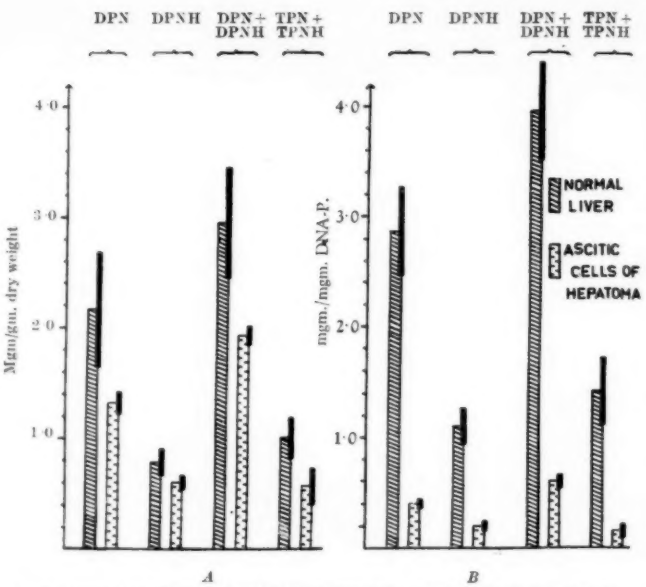


Fig. 2. Comparison of pyridine nucleotide content of normal liver and an ascitic heptoma. *A*, values expressed in mgm. per gm. dry weight; *B*, values expressed in mgm. per mgm. DNA-P. The nuclei of the hepatoma contain on average twice as much DNA as the nuclei of a normal liver

Under the influence of nicotinamide, DPN and reduced DPN increase at about 2–3 times the rate in normal liver as in ascitic cells, while the rate of increase of the triphosphopyridine nucleotides is about twice as great.

The presence of the hepatoma, while affecting the synthesis of ribonucleic acid and DNA of various tissues, as we have shown previously[15,16], does not cause an appreciable fall in the content of di- and triphosphopyridine nucleotides. However, the amounts of pyridine nucleotides in tumour cells retaining the structure of liver trabeculæ are considerably reduced. The most important differences are found at cell level, when referring the values to DNA. Since cell equilibrium can be expressed by the ratio cellular constituent/DNA it is evident that if the quotient, in our case pyridine nucleotide/DNA, is at all modified, it represents a disturbance of this equilibrium.

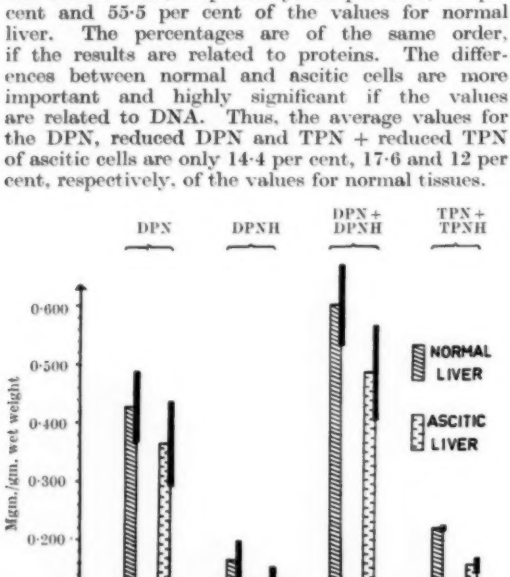


Fig. 1. Comparison of pyridine nucleotide content of normal liver and the liver of an animal with a Zajdela hepatoma

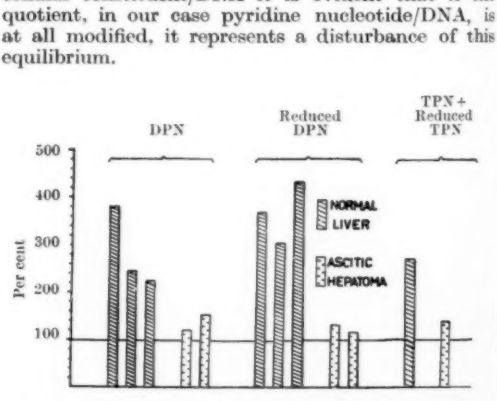


Fig. 3. Effect of injection of 50 mgm. of nicotinamide per 100 gm. wet weight, 8 hr. before killing, on the pyridine nucleotides of a normal liver and the cells of the ascitic hepatoma

The value of 100 was adopted for the controls. The height of the columns which extend above the horizontal line indicates, in per cent, the quantity of pyridine nucleotides in relation to the control

Finally, we find *in vivo* in a homologous tumour a considerable reduction in the net neo-synthesis of di- and tri-phosphopyridine nucleotides after the administration of nicotinamide (Fig. 3).

Summarizing, the reduction of the di- and tri-phosphopyridine nucleotides of an ascitic hepatoma of parenchymatous cells is noticeable when the values are referred to proteins or to the dry weight. The difference is all the more apparent when results are referred to DNA. The synthesis of di- and tri-phosphopyridine nucleotides, after injection of nicotinamide, is much inferior in hepatoma as compared to normal liver. No significant differences in DPN and TPN contents were found between normal liver and the liver of an animal with a hepatoma.

[1] Euler, H., Schlenk, F., Heiwinkel, H., and Högberg, B., *Z. physiol. Chem.*, **256**, 208 (1938).

[2] Bernheim, F., and Felsovanyi, A. V., *Science*, **91**, 76 (1940).

[3] Fischer, A., and Schlenk, F., *Texas Rep. Biol. and Med.*, **6**, 346 (1948).

[4] Carruthers, C., and Suntzeff, V., *Arch. Biochem. Biophys.*, **45**, 140 (1953).

[5] Schneider, W. C., and Hogeboom, G. H., *Cancer Res.*, **11**, 1 (1951).

[6] Wenner, C. E., and Weinhouse, S., *Cancer Res.*, **13**, 21 (1953).

[7] Kensler, C. J., Sugiura, K., and Rhoads, C. P., *Science*, **91**, 623 (1940).

[8] Waravdekar, V. S., Powers, O. H., and Leiter, J., *J. Nat. Cancer Inst.*, **17**, 145 (1956).

[9] Waravdekar, V. S., Powers, O. H., and Leiter, J., *Proc. Soc. Exp. Biol. Med.*, **92**, 797 (1956).

[10] Pitot, H. C., and Potter, V. R., *Biochim. Biophys. Acta*, **40**, 537 (1960).

[11] Holzer, H., Busch, D., and Kröger, H., *Z. physiol. Chem.*, **313**, 184 (1956).

[12] Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, **161**, 83 (1945).

[13] Dische, Z., *Mikrochem.*, **2**, 4, (1930).

[14] Burton, K., *Biochem. J.*, **62**, 315 (1956).

[15] Wintzerith, M., Mandel, L., and Mandel, P., *C.R. Soc. Biol.*, **152**, 2199 (1958).

[16] Wintzerith, M., Klein, N., Mandel, L., and Mandel, P., *Experientia*, **15**, 140 (1959).

BIOCHEMICAL ASPECTS OF RESISTANCE TO 6-MERCAPTOPURINE IN HUMAN EPIDERMOID CARCINOMA CELLS IN CULTURE

By R. W. BROCKMAN, GEORGE G. KELLEY, PATRICIA STUTTS and VIRGINIA COPELAND

Kettering-Meyer Laboratory*, Southern Research Institute, Birmingham, Alabama

THE main pathway by which purines are metabolized to ribonucleotides is by the enzyme-catalysed reactions of the bases with 5-phosphoribosyl-1-pyrophosphate (PRPP) (refs. 1 and 2). Way and Parks[3], and Lukens and Herrington[4] demonstrated that purine analogues react similarly to form nucleotide analogues. A study of the purine ribonucleotide pyrophosphorylase capacity of enzyme preparations from *Streptococcus faecalis* and *L*1210 mouse leukæmia revealed that a specific decrease or loss of inosine 5′-phosphate (IMP) and guanosine 5′-phosphate (GMP) pyrophosphorylase activities accompanied resistance to 6-mercaptopurine (MP) and 8-azaguanine; enzyme preparations from the resistant cells also did not form 8-azaguanosine 5′-phosphate (azaGMP) and MP ribonucleotide in significant amounts[5–7]. Adenosine 5′-phosphate (AMP) pyrophosphorylase activity was not altered in these resistant cell lines.

The present work extends these observations to a human neoplasm in cell culture. The cell line used was the *H.Ep*-2 line derived from a human epidermoid carcinoma[8]; MP-resistant sub-lines of this were developed recently in this Laboratory[9]. The line was grown in static culture on glass and also in suspension culture[10,11]. The resistant sub-lines (*H.Ep*-2/MP) were isolated from clones that developed on glass in the presence of 0·5, 1 and 10 μgm./ml. of MP which, at a concentration of 0·5 μgm./ml., completely inhibited growth of the parent-sensitive line[9]. *H.Ep*-2 cloned in the presence of 1 and 10 μgm./ml. MP grew in suspension cultures in the presence of concentrations of MP as high as 320 μgm./ml. These sub-lines exhibited significant cross-resistance to 8-azaguanine.

The metabolism of purines and analogues was compared in the *H.Ep*-2 cell lines in suspension culture; the cell population per 100 ml. medium at the time of addition of tracer was 40 × 10^6 for *H.Ep*.-2/*S* and 20–30 × 10^6 for the MP-resistant sub-lines. The cells were incubated in the presence of the radioactive substrates for 24 hr., and then soluble extracts of the cells were prepared for two-dimensional paper chromatographic–radioautographic analysis by procedures previously described in studies with ascites tumour cells[5,6]. The residues from the alcohol extractions were treated with hot 10 per cent sodium chloride followed by precipitation of the nucleic acid fraction with ethanol[5,6]. The nucleic acid preparations thus obtained were examined spectrophotometrically and the optical density at the absorption maximum, 260 mμ, was used as a measure of the concentration of nucleic acid. The specific activity of the preparations was determined by evaporation of nucleic acid solutions of known concentration on planchets for counting in internal gas-flow proportional counters.

The results summarized in Table 1 show that adenine extensively labelled the nucleic acids of sensitive and resistant *H.Ep*-2 cells. Guanine, 8-azaguanine and hypoxanthine only labelled the nucleic acids of the sensitive cells. Azaguanine was shown to be incorporated into the ribonucleic acid (RNA) of *H.Ep*-2/*S* as azaguanlyic acid by procedures described by Matthews[12]. The *H.Ep*-2/*S* cells incubated in the presence of 6-mercaptopurine labelled with sulphur-35 were too weakly radioactive for the nature of the material containing sulphur-35 to be identified. Results of analysis of the soluble fractions from these cells showed that nucleotides of adenine but not of guanine, 8-azaguanine, hypoxanthine or 6-mercaptopurine were present in the MP-resistant cells. Conversion of adenine, hypoxanthine and 6-mercaptopurine to nucleotides was extensive in *H.Ep*-2/*S*; the soluble pools of nucleotides derived from guanine and 8-azaguanine were small but detectable.

Enzyme preparations were made from sensitive *H.Ep*-2 cells grown in suspension culture and also from suspension cultures derived from clones which grew in the presence of 0·5, 1 and 10 μgm./ml. of MP. Cells were separated from growth medium by

* Affiliated with the Sloan-Kettering Institute for Cancer Research, New York.

Table 1. Radioactivity of Nucleic Acids from Sensitive and Resistant Human Epidermoid Carcinoma Cells Grown in the Presence of Labelled Purines and Analogues*

Substrate	Specific activity of nucleic acids (counts/sec./mgm.)		
	H.Ep-2/*S*	*H.Ep*-2/MP†	
		1	10
Adenine-8-^{14}C	14,354	17,401	17,086
Guanine-8-^{14}C	1,551	5	2
8-Azaguanine-2-^{14}C	363	3	3
Hypoxanthine-8-^{14}C	9,991	3	4
6-Mercaptopurine-^{35}S	20	2	2

* *H.Ep*-2 cells were grown in suspension culture and incubated in the presence of radioactive substrates for 24 hr. The nucleic acids were isolated for quantitative determinations of radioactivity.

† The two different sub-lines of *H.Ep*-2/MP were isolated on glass by cloning in the presence of concentrations of 6-mercaptopurine of 1 and 10 μgm./ml., respectively.

centrifugation, washed with physiological saline, and re-suspended in cold 0·05 *M* sodium phosphate buffer, *p*H 7·5. The volume of buffer used was 1 ml. for each 4×10^8 cells. The resulting cell suspension was subjected to rapid freezing in an ethanol–dry ice bath followed by gradual thawing; this cycle was repeated twice more to effect disintegration of the cells. The resulting preparation was centrifuged at 95,000*g* for 90 min. at 4° C. in a Spinco centrifuge. The clear supernatant served as the crude enzyme preparation and was frozen and stored at − 15° C. Enzyme solutions thus prepared were analysed for protein by the Oyama–Eagle modification[13] of Lowry's method.

The reaction mixtures used in work on enzyme activities are described in Table 2; they were analysed by methods previously described[5–7]. The results show that enzymes from both the sensitive and the MP-resistant *H.Ep*-2 lines formed nucleotides from adenine, 4-aminopyrazolo(3,4-*d*)pyrimidine, 2,6-diaminopurine, and 4-amino-5-imidazolecarboxamide; in all cell lines the yield of AMP was very much greater than those of these other nucleotides. Conversion of xanthine to xanthylic acid was poor in the sensitive *H.Ep*-2 and was decreased in the MP-resistant lines. Hypoxanthine, MP, guanine, and 8-azaguanine were converted to nucleotides in good yields by enzymes from sensitive cells; yields of these nucleotides with identically prepared enzymes from MP-resistant cells were much lower and in some instances negligible. A decreased capacity for formation of these four nucleotides was evident in all the resistant lines, even in the line cloned in the presence of 0·5 μgm./ml. MP. However, there was some evidence for slight additional loss of capacity in lines cloned at higher concentrations of MP. The cell line cloned in 10 μgm./ml. MP was grown in the absence of MP for 60 generations (*H.Ep*-2/10-MP), after which the loss of enzymic capacity was still essentially complete. This result indicates that resistance to MP probably arose by mutation in the human tumour cell lines, as in other biological systems. The specific loss of IMP and GMP pyrophosphorylase activity in *H.Ep*-2/MP resulted in loss of capacity of the growing cells to form nucleotides of these bases or to incorporate guanine, 8-azaguanine or hypoxanthine into nucleic acids.

The specificity of action of the purine ribonucleotide pyrophosphorylases of *H.Ep*-2 is also of interest. IMP and GMP pyrophosphorylase activities were lost simultaneously, as was the case in MP- and azaguanine-resistant *S. faecalis* and *L*1210[5–7]. This is further evidence that a single enzyme or enzymes closely related genetically[14] may be responsible for this conversion. As in other systems[6,7] MP and azaguanine are evidently metabolized to nucleotides in *H.Ep*-2 by the IMP–GMP pyrophosphorylase enzyme system. Flaks, Erwin and Buchanan[15] observed that 4-amino-5-imidazolecarboxamide and adenine were converted to ribonucleotides by the same pyrophosphorylase. Results of the study of Roy, Haavik and Parks[16] showed that 4-aminopyrazolo(3,4-*d*)pyrimidine and adenine were also converted to ribonucleotides by the same pyrophosphorylase. (These workers found that 20-fold purified AMP pyrophosphorylase from beef liver was active in catalysing reactions of PRPP with 4-aminopyrazolo(3,4-*d*)pyrimidine, 2-fluoro-adenine, 2-aza-adenine and 8-aza-adenine.) Results of the present work are compatible with these results and suggest that in *H.Ep*-2 cells adenine, 4-aminopyrazolo(3,4-*d*)pyrimidine and 4-amino-5-imidazolecarboxamide are acted on by AMP pyrophosphorylase which is clearly distinct from IMP–GMP pyrophosphorylases. (Growth of MP-resistant *H.Ep*-2 cells was inhibited by 4-aminopyrazolo(3,4-*d*)-pyrimidine. Similarly, Skipper *et al.*[17], and Venditti, Frei and Goldin[18] observed that growth of 8-azaguanine-resistant *L*1210 tumours was inhibited by 4-aminopyrazolo(3,4-*d*)pyrimidine; this resistant neoplasm also retained AMP pyrophosphorylase activity while showing significant decreases in IMP–GMP pyrophosphorylase activities[5,6].) Similarly, Lieberman and Ove[19] studied resistance to purine analogues in mammalian cells in culture and observed that a 2,6-diaminopurine-resistant sub-line showed specific

Table 2. Conversion of Bases to Ribonucleotides by Pyrophosphorylases from 6-Mercaptopurine-Sensitive and -Resistant Human Epidermoid Carcinoma (*H.Ep*-2) in Cell Culture

Ribonucleotide formed	mμmoles ribonucleotide formed per mgm. protein in 1 hr.*				
	H.Ep-2 (sensitive)	MP-resistant *H.Ep*-2†			
		0·5	1·0	10	10-MP‡
Adenylic acid	414	576	552	429	359
4-Aminopyrazolo-3,4-*d*)-pyrimidine ribotide	67	21	14	71	76
2,6-Diaminopurine ribotide	72	10	22	58	43
5-Amino-4-imid-azolecarboxamide ribotide	72	58	32	60	67
Xanthylic acid	21	3	7	1	1
Inosinic acid	839	18	< 1	< 1	< 1
6-Mercaptopurine ribotide	766	16	2	< 1	< 1
Guanylic acid	383	13	14	4	1
8-Azagunaylic acid	243	5	7	6	1

* The reaction mixture consisted of base (0·0005 *M*; 0·2 μc. per ml.), magnesium PRPP (0·002 *M*), and enzyme (approximately 200 μgm. of protein per ml. incubation mixture) in 0·1 *M tris* buffer, *p*H 7·6. Incubation time was 1 hr. at 37° C.; enzyme action was stopped and reaction mixtures deproteinized by heating for 5 min. at 100° C. Aliquots (0·1 ml.) were analysed by descending chromatography on Whatman 3 *MM* paper using 70 per cent *iso*propanol in an ammonia atmosphere to separate bases from the corresponding ribonucleotides. Radioactive areas of chromatograms located by radioautography were eluted with water; the eluates were evaporated on steel planchets and quantitative determinations of radioactivity were made by counting in gas-flow proportional counters. For identification purposes bases, ribonucleosides and ribonucleotides were separated and identified by two-dimensional chromatography and by paper electrophoresis using techniques already described (refs. 5–7).

† Concentration of MP (μgm./ml.) in which cell lines were originally cloned and isolated on glass. The clones developing in 0·5, 1·0 and 10 μgm./ml. of MP were subcultured in the presence of these concentrations of MP, forming confluent monolayers of cells. These resistant cell lines were then grown in suspension culture to provide material for enzyme preparations.

‡ *H.Ep*-2/MP cloned in 10 μgm./ml. of MP was resistant to concentrations of MP approaching the solubility limit of the inhibitor (320 μgm./ml.). This line was then grown in the absence of MP for 60 generations, after which enzyme preparations were then assayed for purine ribonucleotide pyrophosphorylase activity with the results shown in this column.

loss of AMP pyrophosphorylase activity; they suggested that 2,6-diaminopurine was anabolized by AMP pyrophosphorylase. This would also be consistent with the results obtained here with *H.Ep-2*.

Decreased activity of enzymes involved in the formation of nucleotides has now been observed to accompany the development of resistance to purine analogues in a variety of biological systems. Similar observations have been made in studies of resistance to pyrimidine analogues in both mammalian cells[20–23] and in micro-organisms[24,25]. Such alterations of enzyme activities may be of genetic origin since in many cases these losses have been demonstrated to be stable heritable characteristics of the resistant cells. The development of resistance to metabolite analogues is a serious problem in the therapy of neoplastic diseases[26], and an understanding of the nature and mechanism of the biochemical changes leading to resistance to metabolite analogues poses fundamental questions in areas of enzymology and biochemical genetics.

The work was supported by grants from the Charles F. Kettering Foundation and the Alfred P. Sloan Foundation, and by contract No. *SA-43-ph-2433*, Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health.

[1] Korunberg, A., Lieberman, I., and Simms, E. S., *J. Biol. Chem.*, **215**, 417 (1955).
[2] Korn, E. D., Remy, C. N., Wasilejko, H. C., and Buchanan, J. M., *J. Biol. Chem.*, **217**, 875 (1955).
[3] Way, J. L., and Parks, jun., R. E., *J. Biol. Chem.*, **231**, 467 (1958).
[4] Lukens, L. N., and Herrington, K. A., *Biochim. Biophys. Acta*, **24**, 432 (1957).
[5] Brockman, R. W., Bennett, jun., L. L., Simpson, M. S., Wilson, A. R., Thomson, J. R., and Skipper, H. E., *Cancer Res.*, **19**, 856 (1959).
[6] Brockman, R. W., *Cancer Res.*, **20**, 643 (1960).
[7] Brockman, R. W., Debavadi, C. S., Stutts, P., and Hutchison, D. J., *J. Biol. Chem.*, **236**, 1471 (1961).
[8] Moore, A. E., Sabachewsky, L., and Toolan, H. W., *Cancer Res.*, **15**, 598 (1955).
[9] Kelley, G. G., Vail, M. H., Adamson, D. J., and Palmer, E. H., *Amer. J. Hyg.*, **73**, 231 (1961).
[10] Kelley, G. G., Adamson, D. J., and Oliver, K. L., *Amer. J. Hyg.*, **71**, 9 (1960).
[11] Kelley, G. G., Adamson, D. J., and Vail, M. H., *Amer. J. Hyg.*, **72**, 275 (1960).
[12] Matthews, R. E. F., *J. Gen. Microbiol.*, **10**, 521 (1954).
[13] Oyama, V. I., and Eagle, H., *Proc. Soc. Exp. Biol. Med.*, **91**, 305 (1956).
[14] Anderson, E. P., and Law, L. W., *Ann. Rev. Biochem.*, **29**, 577 (1960).
[15] Flaks, J. G., Erwin, M. J., and Buchanan, J. M., *J. Biol. Chem.*, **228**, 201 (1958).
[16] Roy, J. K., Haavik, C. A., and Parks, jun., R. E., *Proc. Amer. Assoc. Cancer Res.*, **3**, 146 (1960).
[17] Skipper, H. E., Montgomery, J. A., Thomson, J. R., and Schabel, jun., F. M., *Cancer Res.*, **19**, 425 (1959).
[18] Venditti, J. M., Frei, E., and Goldin, A., *Cancer*, **13**, 959 (1960).
[19] Lieberman, I., and Ove, P., *J. Biol. Chem.*, **235**, 1765 (1960).
[20] Reichard, P., Sköld, O., and Klein, G., *Nature*, **183**, 939 (1959).
[21] Heidelberger, C., Ghobar, A., Baker, R. K., and Mukherjee, K. L., *Cancer Res.*, **20**, 897 (1960).
[22] Brockman, R. W., and Law, L. W., *Proc. Amer. Assoc. Cancer Res.*, **3**, 98 (1960).
[23] Pasternak, C. A., Fischer, G. A., and Handschumacher, R. E., *Cancer Res.*, **21**, 110 (1961).
[24] Handschumacher, R. E., and Pasternak, C. A., *Biochim. Biophys. Acta*, **30**, 451 (1958).
[25] Brockman, R. W., Davis, J. M., and Stutts, P., *Biochim. Biophys. Acta*, **40**, 22 (1960).
[26] Skipper, H. E., Heidelberger, C., and Welch, A. D., *Nature*, **179**, 1159 (1957).

LIMITS OF HUMAN VISION

By Dr. R. A. WEALE

Department of Physiological Optics, Institute of Ophthalmology, Judd Street, London, W.C.1

FUNDUS reflectometry provides a means for studying the properties of visual pigments in the living eye. Whereas the detection of cone pigments is easy[1,2], the study of the human extra-foveal regions is complicated by their mixed receptor population. A detailed study of these regions[3] has shown that the unequivocal separation of rod and cone effects from each other is unlikely to be achieved without a close examination of difference spectra[4]. However, in the green region of the spectrum (510–520 mμ), where bleaching the dark-adapted eye with white light produces a maximum density difference, $\Delta D_{\lambda\max.}$, the effect of rod visual purple may be presumed to be dominant. If the bleaching intensity is expressed in the same units in which the absolute threshold is measured in stated conditions, an attempt to correlate the two types of measurement becomes feasible, all the more so as one is dealing with one species only, namely, man.

The latest model of the fundus reflectometer[2] was used, two retinæ being examined at locations shown in Table 1. Dark-adaptation preceding bleaching measurements always continued for 20 min., but 30 min. in the absolute threshold measurements. The bleaching exposure lasted 30 sec., the intensity being varied as shown along the abscissa (Fig. 1). The threshold measurements involved a criterion of 50 per cent 'seen' of the bleaching light, exposed for 0·036 sec. and reduced from 3 to 1·7° angular diameter.

The changes in density, $\Delta D_{\lambda\max.}$, are plotted on a percentage basis (Fig. 1) as a function of bleaching intensity. The maximum values corresponding to each subject and the retinal positions are shown in Table 1 and represent values for double transit through the retina. $\Delta D_{\lambda\max.}$ rises with intensity to a maximum at log scotopic trolands = 6·5.

A comparison of the bleaching and threshold data with work of Aguilar and Stiles[5] is of interest. These workers measured accurately the incremental threshold of rods (ordinate) as a function of adapting field luminance (abscissa). Their mean curve is reproduced in Fig. 1. The average of the present threshold measurements agrees with theirs as well as can be expected in view of the difference between the experimental conditions. But the following is the focal point of interest in the comparison. Aguilar and Stiles estimate that, in the region indicated with the arrowed bracket, human rods are saturated. This is to say, no matter how great the field intensity, the increment required to make the test visible approaches infinity. They calculate that this saturation is unlikely to be

Table 1. Maximum Values Corresponding to each Subject and Retinal Positions*

	A $\Delta D_{\max.}$		B $\Delta D_{\max.}$
8° *N* ret.	0·093	◑	0·116
10° *T* ret.	0·095	◧	0·150
13° *T* ret.	0·125	▼	0·130
17° *T* ret.	0·085	▲	0·157

* This refers to Fig. 1.

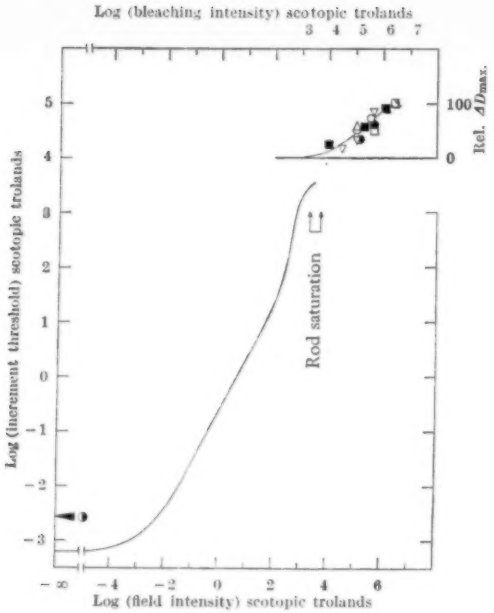

Log (bleaching intensity) scotopic trolands
3 4 5 6 7
100
0
Rel. ΔDmax.
Rod saturation
Log (increment threshold) scotopic trolands
5 4 3 2 1 0 −1 −2 −3
−∞ −4 −2 0 2 4 6
Log (field intensity) scotopic trolands

Fig. 1

due to the exhaustion of visual purple. The present results bear out this expectation: in the region of rod saturation the amount of pigment bleached becomes just measurable and is exhausted only at intensities nearly one thousand times as high.

The present results can be related to the absolute threshold in another way. Pirenne has shown in a very interesting paper[4] that visual purple would be expected to bleach at the rate of 1 per cent/sec. when the bleaching intensity is 10^{10} threshold. At first sight the results shown in Fig. 1 appear to contradict this prediction, such a bleaching rate occurring at about 10^7 threshold. But the following argument shows that the present results do, in fact, come close to Pirenne's prediction that the threshold and the rate of bleaching can be related along the lines which he suggested.

Let Q be the number of quanta absorbed at the absolute threshold; t, the time of exposure of the threshold test-light; A, its angular area in square degrees; N, the number of rods per square degree[6]; ν, the number of chromophores per rod[6]; α, the effective percentage absorption of the visual pigment[6], p, the fraction of pigment bleached in T, the bleaching time; R, the ratio (the energy flux per unit time bleaching p per cent of pigment) ÷ (the flux producing the threshold response); γ, the quantum efficiency (number of chromophores bleached ÷ quanta absorbed); D, the orientation factor[8]; then:

$$p = \frac{RTQ\gamma D}{\alpha ANt\nu}$$

The following are among important assumptions in the derivation of this expression: absorption leads to bleaching; the amount bleached is such as not to change the absorption to a very marked extent (a proviso obeyed approximately in present conditions when $p = 0{\cdot}2$); in the conditions of the experiment, the quantum efficiency is approximately 0·5 (refs. 4, 7); blinking[9] is not very important. Denton[8] has shown that the absorbing molecules are so positioned as to render absorption maximal when the light travels along the axis of the rod. It is doubtful whether this can apply in all rigour to the human eye, the rods pointing approximately at the posterior nodal point, and it will apply with even less certainty to light re-traversing the retina after reflexion from the fundus. But accepting a value of $D = 1{\cdot}5$ for this effect and a quantum efficiency of $\gamma = 0{\cdot}5$, the number of chromophores ν has to be multiplied by 1·33. Substitution of experimental values in the equation leads to $Q = 7$–14 if some uncertainty in the results is allowed for. The calculated value yields a result of the order of magnitude obtained by other methods[6]. Although ν, the number of chromophores per rod, was calculated on the basis of frog visual purple data[6], it is unlikely that comparably accurate data for human visual purple would affect the situation very much[10].

An increase in bleaching intensity beyond that shown in Fig. 1 leads to an unexpected result. The change in density recorded for the wave-length of maximum change is smaller at the highest intensity used (cf. Fig. 2) than it is at the top intensity shown in Fig. 1. This finding appears to hold for both extra-foveal and foveal parts of the retina. Control of the pupil diameter[3,11] reduces the likelihood of miosis of the strongly mydriaticized iris being responsible for this effect in intense illumination. An extension of the recording of changes of density to the whole of the visible spectrum shows that the apparent reduction in $\Delta D_{\lambda max.}$ is due to an upward move of the whole base-line, a finding which can be pictured by the hypothesis that, in very intense illumination, a grey filter is formed in the eye. The effect disappears after dark-adaptation for 3 min. An early stage in thermal lesions, known to lead to retinal œdema, is manifested by a greying of the fundus oculi. The view that the rise in the zero line mentioned above is an even earlier manifestation of such a process is supported by radiometric measurements of the intensity of the bleaching light (top of Fig. 2). These

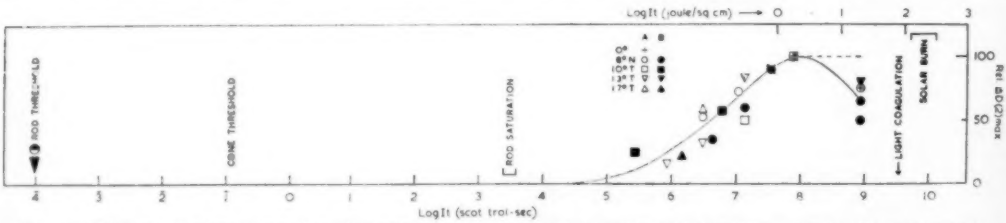


Fig. 2. Ordinate: Relative values of maximum change of density (double transit through retina) in various locations of two dark-adapted human retinæ *A* and *B* (see inset). Abscissa: bleaching intensity × exposure time in scotopic trolands × sec. (bottom); in joules/sq. cm. on the retina (top). Free-hand curve. Effects due to blinking and absorption in pre-retinal media are neglected. The rod threshold was measured on subjects *A* and *B* (cf. Fig. 1). The broken line represents the function of $\Delta D(2)_{max.}$ when the œdema effect is compensated

relate the present results to exposures causing solar burns[12], just as an extension of the photometric scale (bottom of Fig. 2) relates them to exposures leading to light-coagulation[13]. It may be suggested tentatively that the constitution of human retinal light-sensitive material is such that the lower limit of the visual range is governed by quantum physics, whereas the upper limit coincides with the safety-level of ocular tissues exposed to thermal hazards.

[1] Weale, R. A., *J. Physiol.*, **127**, 587 (1955).
[2] Weale, R. A., *Optica Acta*, **6**, 158 (1959).
[3] Weale, R. A., *Vision Res.* (in the press).
[4] Dartnall, H. J. A., *The Visual Pigments* (Methuen and Co., London, 1957).
[5] Stiles, W. S., and Aguilar, M., *Optica Acta*, **1**, 59 (1954).
[6] Pirenne, M. H., *Biol. Rev.*, **31**, 194 (1956).
[7] Hagins, W. A., *J. Physiol.*, **129**, 22*P* (1955).
[8] Denton, E. J., *J. Physiol.*, **124**, 16*P* (1954).
[9] Lawson, R. W., *Nature*, **162**, 531, 970 (1948).
[10] Dartnall, H. J. A., *N.P.L. Symposium on Visual Problems of Colour*, Paper No. 3 (H.M.S.O., 1958).
[11] Weale, R. A., *Amer. J. Ophthal.* (in the press).
[12] Duke-Elder, W. S., *Text-book of Ophthalmology*, **6** (Henry Kimpton, London, 1954).
[13] Meyer-Schwickerath, G., *Arch. Ophthal.*, **156**, 2 (1955).

PHOSPHATASES IN *SCHISTOSOMA MANSONI*

By Dr. D. L. H. ROBINSON

Medical Research Council Group for Research in Bilharzia, Winches Farm, St. Albans

Distribution of Alkaline Phosphatase

DUSANIC[1] made a histochemical study of alkaline phosphatase in larval and adult *Schistosoma mansoni* and tissues associated with these stages in both molluscan and final hosts. In adult flukes the enzyme was found in the subcuticular cells, the mesenchyme, the ovaries, testes and the intestinal cells. High concentrations of the enzyme were found in the intestinal cells, and in the ovaries and testes alkaline phosphatase activity was considerably greater than in the mesenchymal cells.

During an examination of the methods of carbohydrate uptake in schistosomes, I investigated the distribution of alkaline phosphatase in *S. mansoni*. Mature flukes were obtained from the portal and mesenteric veins of hamsters 8–10 weeks after infection with 150 cercariæ of an Egyptian strain of the schistosome maintained in a Puerto Rican strain of *Australorbis glabratus*. After a quick rinse in cold saline, the worms were transferred as rapidly as possible to ice-cold acetone. Alkaline phosphatase activity was determined by Glick's[2] modified Gomori method using a sodium glycerophosphate substrate. Sections cut in both longitudinal and transverse planes at 10μ were incubated at 37° C. for 1 hr., this time having been arrived at by the method of Martin and Jacoby[3]. Control slides, incubated without substrate, were negative.

Fig. 1 shows part of a transverse section of a male *S. mansoni* in the region of the testis. High alkaline phosphatase activity is present in the subcuticular cells and in the mesenchyme, but considerably less in the testis. The black deposit in one of the cæcæ in Fig. 1 is due to pigment in the lumen, but the intestinal cells show little activity except for the nuclei. Similar results were obtained in the intestinal cells of female worms and the ovaries exhibited lower activity than the mesenchyme.

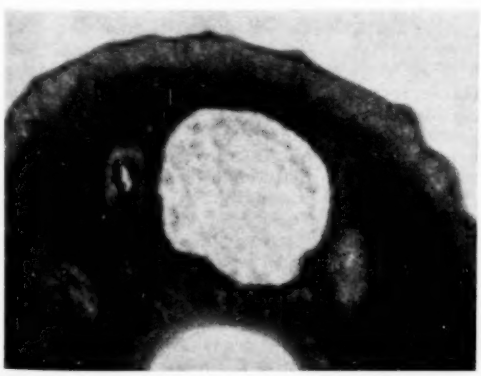


Fig. 1. Portion of a transverse section of a male *Schistosoma mansoni* showing distribution of alkaline phosphatase

There appears to be abundant evidence available to confirm the view of Rogers[4] that regions exhibiting high alkaline phosphatase activity might be associated with carbohydrate absorption. Based on this hypothesis, the results given above, in contrast to those of Dusanic[1], suggest that the major region of carbohydrate uptake from the environment in *S. mansoni* is the cuticle, with the intestinal cells playing a secondary, if not a minor part.

Quantitative Estimation of Phosphatases

Histochemical examination shows that high alkaline phosphatase activity is present in *Schistosoma mansoni* at *p*H 9·4. Dusanic[1] observed that the alkaline phosphatase had a *p*H optimum of 9·0–9·5, and exhibited only slight activity at *p*H 8·6. At *p*H 8·0 no activity could be detected by histochemical methods.

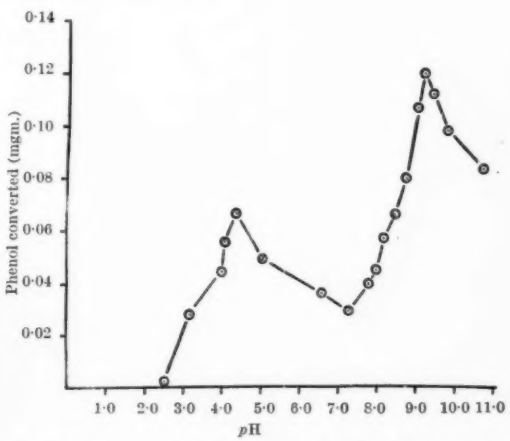


Fig. 2. Phosphatase activity of extract of male *S. mansoni* at different hydrogen ion concentrations. 130 worms, extract made up to 25 ml. Incubation time, 18 hr.

XUM

The quantitative experiments reported here were performed: (*a*) to determine the *p*H optima of the alkaline phosphatase of *S. mansoni*; (*b*) to demonstrate an acid phosphatase if one were present; (*c*) to compare the activity of these enzymes, at different hydrogen ion concentrations.

Mature schistosomes were removed from the portal veins of hamsters infected 8 weeks previously with *S. mansoni*. Pairs were separated and male and female worms counted and ground separately in a glass tube containing distilled water. Toluene was then added and the mixture allowed to stand on the bench for 4–5 hr. After centrifugation the supernatant was stored overnight in the refrigerator. The solution was diluted immediately before use.

Incubation mixtures consisted of 1 ml. substrate, 1 ml. extract, 1 ml. buffer and 0·5 ml. toluene. The buffers used were acetate/barbiturate/hydrochloric acid (Michaelis 1931) and glycine/sodium hydroxide (Sorensen Walbum, 1920), and the substrate was 0·01 *M* disodium monophenyl phosphate. The *p*H of each mixture was determined, before and after incubation, on a Pye *p*H meter. After incubation, aliquots were removed for estimation of the phenol liberated, by the method of King and Armstrong[5], using the reagent of Folin and Ciocalteau. Controls consisted of unincubated mixtures, and the results were deducted from those of the test.

An alkaline and an acid phosphatase were present in male and female worms. No sex differences were observed in their characteristics. The alkaline phosphatase had its optimum in the region of *p*H 9·3 and the acid phosphatase had an optimum in the region of *p*H 4·4. As can be seen from Fig. 2, the alkaline phosphatase exhibited the higher relative activity.

Bueding[6] found that carbohydrate uptake in *S. mansoni* amounted to the equivalent of one-fifth of its dry weight per hour. The high alkaline phosphatase activity reported above may therefore be linked with this high carbohydrate uptake and adds further support to the view of Rogers[4] that regions of high alkaline phosphatase activity are associated with carbohydrate absorption.

[1] Dusanic, D. G., *J. Infect. Dis.*, **105**, 1 (1959).

[2] Glick, D., *Techniques of Histo- and Cytochemistry* (Interscience, New York, 1949).

[3] Martin, B., and Jacoby, F., *J. Anat.*, **83**, 351 (1949).

[4] Rogers, W. P., *Nature*, **159**, 374 (1947).

[5] King, E. J., and Armstrong, A. R., in *Chemical Methods in Clinical Medicine*, edit. Harrison, G. A. (J. and A. Churchill, London, 1947).

[6] Bueding, E., "Metabolism of Helminths", from *Parasitic Infections in Man*, edit. Most, H., 150 (Columbia Univ. Press, New York, (1951).

SOCIAL DOMINANCE IN INBRED MOUSE STRAINS

By Prof. G. LINDZEY, H. WINSTON and M. MANOSEVITZ

Department of Psychology, University of Minnesota, Minneapolis 14

ORIGINS of the experimental study of temperament in lower animals[1–3] are approximately as remote as origins of the experimental study of learning in lower animals[4,5]. In spite of this historical similarity the two domains to-day are dramatically different in their respective achievements. By almost any reasonable criterion the area of learning has attained a state of maturity far surpassing that of research in animal temperament. While there are undoubtedly many factors that have contributed to this relative deficiency in investigation of animal temperament, one of the most important of these is the absence of a set of sensitive and reliable measures that can be used to assess variables commonly viewed as significant or interesting.

This article is presented as a very small corrective to this deficiency. It describes a measure of social dominance and presents tentative results, based on the comparison of three inbred strains of mice, suggesting that the test possesses reasonable sensitivity and stability. For present purposes we may define social dominance simply as the tendency of an animal to make a goal response when this response involves overcoming the competing response of another animal similarly motivated.

The subjects in this work consisted of 44 animals in each of three inbred mouse strains. Breeding stock for each of the strains was obtained from the colony maintained by Prof. John Bittner in the Department of Cancer Biology at the University of Minnesota. Mice within each strain have been bred by brother–sister mating well beyond the twenty generations that are conventionally believed to lead to offspring that are approximately homozygous. The three strains and the number of generations of inbreeding are as follows: *A*/alb (57), *C3H* (97), *DBA*/8 (28). The subjects were at least 60 days old, and animals that were opposed to each other were matched in age and sex. All animals were individually housed after weaning at 22 days of age.

The dominance test consists of a 'Lucite' tube, 1 in. inside diameter and 17 in. long. The tube is connected at each end to a goal box that is 4 in. × 4 in. × 2 in. and covered by a wire cloth top. Guillotine doors are located at the entrance to each goal box and a small 'Lucite' divider can be inserted midway in the tube. The diameter of the tube is just sufficient to permit a mature mouse to pass through it readily, but not sufficient to permit two mice to pass one another in the tube. It should be mentioned that although this technique was devised in our laboratory, after we had worked with it for a period of time, we learned that Prof. Tsai of Tulane University had used a similar apparatus with rats. Each animal was given a series of five training trials on each of three successive days. The animals were run, deprived of food (85 per cent of normal weight) with the reward of wet mash in the goal box after they had passed through the tube. During the training trials the mice were alternated in the direction that they ran through the tube and the experimenter recorded the time required to reach the goal box on each trial. Following this, there was a period of five dominance test days on each of which the animals received individually three further training trials before the test trials.

On a dominance trial the mice were placed at the same time in two goal boxes at the entrances to the tube and when they had both reached the centre of

the 'Lucite' tube the divider was removed and the issue was joined. The trial ended when one mouse had forced the other to retreat with all four feet into the goal box from which it had commenced. The order of the various oppositions within each group, and the goal box in which each mouse began, were systematically varied on successive days. The animals were deprived of food during all the test trials.

Mice were run in groups of four. Each group included two animals from two of the three strains under investigation and each mouse opposed every other mouse in his group on each of the five test days. Thus, there were eleven groups involved in each of the three strain comparisons (*A*/alb versus *C3H*, *A*/alb versus *DBA*/8, *C3H* versus *DBA*/8). We were interested in differences of strain and for each mouse there was a dominance opposition to two mice from the other strain on each test day, or a total of ten such oppositions per mouse by the end of the test series.

The simplest and most meaningful indexes of *stability* consist of the proportion of mice that achieved dominance by winning all five of their trials, the proportion that achieved dominance by winning four out of five trials, and the proportion that won three out of five trials. In the opposition between *A*/alb and *C3H* mice, 57 per cent of the dominant mice won all five trials, 27 per cent won four trials, and 16 per cent won three trials. In the opposition between *A*/alb and *DBA*/8 mice, 75 per cent of the dominant animals won all five trials, 20 per cent won four trials, and 5 per cent won three trials. In the opposition between *C3H* and *DBA*/8 mice, 64 per cent of the dominant animals won all five trials, 18 per cent won four trials, and 18 per cent won three trials. If we combine the results for all strain comparisons, we find that in approximately two-thirds of the cases, there was complete consistency, with the same animal winning all five of the trials. In slightly more than one-fifth of the oppositions the dominant animal won four out of the five trials, and in less than one-eighth of the cases the dominant animal won only three out of the five trials. In general, the consistency across repeated trials of the test was so marked that, knowing the performance of a particular pair of mice for a single trial, provides an excellent basis for predicting the outcome of the four subsequent trials.

The matter of *transitivity* is somewhat more difficult to represent although the results for strain comparisons are obvious. There is complete transitivity between strains, for example, *A*/alb > *C3H*, *C3H* > *DBA*/8, *A*/alb > *DBA*/8; moreover, the fact that the superiority of the *A*/alb over the *DBA*/8 is greater than the superiority of the *A*/alb over the *C3H* is reflected in the dominance of the *C3H* strain over the *DBA*/8 strain. Nevertheless, there are so few strain comparisons possible in our work that this is not altogether a satisfactory measure.

An index of transitivity between individual animals was devised that could be applied to each group of four animals. A group was declared transitive if it arranged itself in an hierarchical manner, so that the most dominant subject dominated all other animals, the second most dominant animal dominated all animals except the most dominant, and so on. Using this procedure there were a total of 33 such groups and all but two met this stringent criterion of transitivity. Thus, our evidence suggests a high degree of transitivity in the dominance relations of individual animals as well as in the relations between strains.

Table 1. STRAIN DIFFERENCES IN DOMINANCE

	A/alb versus *C3H*		*A*/alb versus *DBA*/8		*C3H* versus *DBA*/8	
Per cent of total trials dominant *n* (animals) = 22 *n* (observations) = 220	78	22	94	6	78	22
Per cent of total pairings dominant *n* (animals) = 22 *n* (observations) = 44	84	16	98	2	82	18
Per cent of animals winning more than one-half of the oppositions with other strain *n* (animals) = 22 *n* (observations) = 22	86	14	100	0	86	14

Previous investigators who have been concerned with measuring dominance in animals have not, for the most part, concerned themselves with the reliability or transitivity of their measures. Those investigators who have inquired into those questions[6-8] have reported results indicating much less stability than we have observed.

The results summarized in Table 1 leave no doubt about the existence of *differences between strains* in performance on this test. The results of the strain oppositions are summarized by means of three correlated indexes that are completely consistent in the direction and the magnitude of the results they depict. Perhaps the most revealing of these indexes is the third, which summarizes the number of animals within each strain that won more than half of their trials against animals from the comparison strain. The results indicate that 86 per cent of the *A*/alb mice won more than half of their oppositions against the *C3H* mice, 100 per cent of the *A*/alb mice won more than half their oppositions against the *DBA*/8 mice, and 86 per cent of the *C3H* mice won more than half of their oppositions against the *DBA*/8 mice. Thus, animals in the *A*/alb strain are dominant over animals in the *C3H* and *DBA*/8 strains and animals in the *C3H* strain are dominant over animals in the *DBA*/8 strain. Moreover, even given the genetic diversity of these strains, the magnitude of these differences is unusually large when compared with results obtained with other behavioural measures.

Results of this nature obviously suggest the importance of gene factors in determining dominance behaviour as we have measured it. There seems little doubt that a considerable proportion of the variance in our measure can be attributed to variation in genetic factors. These results are consistent with those reported by Ginsburg and Allee[9] on the basis of observing fighting behaviour in different strains. Uyeno[10], using a food competition measure of dominance, in conjunction with a single generation of selective breeding, also found evidence for the contribution of gene factors to dominance behaviour.

A further result of such a clear-cut differentiation between strains is some increase in our confidence in the measure we are employing. In other words, when subjects known to be quite distinctive in behaviour[11,12] are compared in terms of a particular measure, our interest in this measure is considerably dampened if it proves unable to discriminate between these distinctive organisms. Conversely, when the test can discriminate among subjects with unusual effectiveness, our confidence that the test is measuring something significant is increased.

XUM

Given that we have a measure that is rationally linked to dominance, and that it differentiates successfully between subjects that are known to be different in behaviour, there still remain many alternative explanations to be explored before we can be content with the label we have attached to the measure. For example, one might reason that the differences we have observed between strains are attributable to some simple, extraneous factor that may be genetically controlled and at the same time serves to influence performance on this test. It may be, for example, that the strains differ widely in weight so that even when animals are matched in age and deprivation experience certain strains will have an appreciable advantage in weight, and this difference will determine the result of the test procedures. This possibility was explored both within strains and between strains and we found no evidence for an association between weight and dominance. A second alternative hypothesis suggests that the strains may differ in the effectiveness with which they learn the original task involving passage through the tube and, consequently, they may be differentially motivated when placed in opposition in the tube. This possibility was examined by relating performance during the training trials (average time per trial) to performance on the dominance measure, but again we found no evidence of any association between the two variables. Thus, two of the most evident means of weakening the inferential link between the concept of dominance and our measure were found to be inconsistent with available evidence.

It is obvious that future investigations exploring the relation between the present measure of dominance and other measures of the same variable are very much needed. Our preliminary results indicate important differences in the results obtained with our measure and with the food competition measure employed by Rosen[13] and Uyeno[10]. It is also important that the relation between dominance and such related temperament variables as aggression and fearfulness should be explored. Given the results of such research, in addition to more detailed information concerning the determinants of performance on our measure, it should be possible to arrive at a meaningful estimate of the significance of this device for the student of animal temperament.

This work was supported by a grant from the Ford Foundation and Research Grant *M*–3536 from the National Institute of Mental Health of the National Institutes of Health, U.S. Public Health Service.

[1] Berry, C. S., *J. Comp. Neur. Psychol.*, **16**, 333 (1906).
[2] Stewart, C. C., *Amer. J. Physiol.*, **1**, 40 (1898).
[3] Yerkes, R. M., *J. Anim. Behav.*, **3**, 286 (1913).
[4] Small, W. S., *Amer. J. Psychol.*, **11**, 80 (1900).
[5] Thorndike, E. L., *Psychol. Monogr.*, **2**, No. 8, 109 (1898).
[6] Ross, S., and Berg, J., *J. Mammal.*, **37**, 129 (1956).
[7] Ross, S., and Scott, J. P., *J. Comp. Physiol. Psychol.*, **42**, 75 (1949).
[8] Seward, J. P., *J. Comp. Psychol.*, **38**, 213 (1945).
[9] Ginsburg, B., and Allee, W. C., *Physiol. Zool.*, **15**, 485 (1942).
[10] Uyeno, E. T., *J. Comp. Physiol. Psychol.*, **53**, 138 (1960).
[11] Lindzey, G., *J. Comp. Physiol. Psychol.*, **44**, 389 (1951).
[12] Lindzey, G., Lykken, D. T., and Winston, H. D., *J. Abn. Soc. Psychol.*, **61**, 7 (1960).
[13] Rosen, J., *Canad. J. Psychol.*, **12**, 229 (1958).

SIGNIFICANCE OF THE ANTI-SPORULATION EFFECT OF COPPER FUNGICIDES IN THE CONTROL OF SIGATOKA DISEASE OF BANANAS CAUSED BY *MYCOSPHÆRELLA MUSICOLA* LEACH

By E. EVANS, H. L. S. BEVINGTON and E. P. WHITAKER

Fisons Pest Control, Ltd., Chesterford Park Research Station, Nr. Saffron Walden

IT is now a well-established belief that all copper fungicides are protective in function when used for the control of plant diseases. As such, an attempt is always made to cover the most susceptible parts of the host plant with a layer of chemical prior to the onset of the disease epidemic. This was the standard practice for the control of Sigatoka disease of bananas in most parts of the world until the introduction of eradicant oil spraying in 1956[1]. Bordeaux mixture is still being used in many parts of the world, but this practice is gradually becoming less economic. Accordingly, attempts have been made to introduce the ultra-low-volume application of copper fungicides from the air and from ground machines.

For the past six years we have been closely associated with the aerial application of copper fungicides to a number of crop plants, more particularly to potatoes and bananas. During visits to the field in West Africa and Central America, as well as from greenhouse studies in the United Kingdom, it has become obvious that the banana plant has unusual physical characteristics from the point of view of the retention of spray droplets. Leach[2] has shown that the spike, first and second open leaves are those most susceptible to leaf spot, yet a simple survey of any air-sprayed area of bananas shows quite clearly that these are rarely adequately covered with chemical. One of the main reasons for this phenomenon appears to be the highly reflective characteristics of these leaf surfaces as well as the exceptionally rapid development of the spike leaf during the main growing season.

From observations such as these it seems reasonable to suggest that any aerial application of a protective fungicide would not be particularly successful in controlling the disease unless such a chemical also acted by some other mechanism. One obvious alternative would be an anti-sporulation effect[3].

By courtesy of the United Fruit Co., laboratory facilities were made available in 1960 to two of us at Palmar Sur in Costa Rica (E. E., H. L. S. B.). During this visit an attempt was made to evaluate a number of standard fungicides as anti-sporulants, using a modification of the method recommended by R. W. Fulton (personal communication). Basically, this consisted of collecting leaf segments from the field, with Sigatoka lesions at the correct stage of

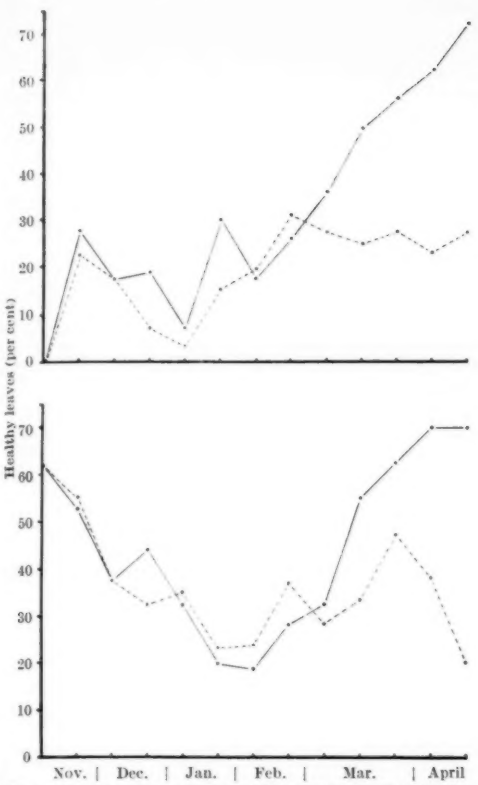


Fig. 1. Percentage of clean and lightly infected leaves in treated and untreated plots on parent (above) and follower (below) plants in a mature stand of bananas. ——, copper oxychloride; – – –, untreated control

development, spraying these lesions with the test chemicals and incubating for 24 hr. at 26° C. prior to examination under a stereoscopic microscope. The evaluations were made by comparing the number of sporulating lesions in the treated samples with those in the untreated controls. Sample data obtained in this manner are given in Table 1.

Table 1 shows quite clearly that standard fungicides such as copper oxychloride, zineb and a mixture of these two preparations have a marked effect on conidial production of lesions of *Mycosphærella musicola*. Since any lesion which produced a single recognizable spore was classified as a sporulating lesion, the results vastly under-estimate the total reduction of spores produced by the treated over the untreated lesions.

At the same time as these experiments were being carried out in Costa Rica, one of us (E. P. W.) was carrying out small-scale low-volume field trials on

Table 1. Anti-sporulation Effects of Certain Standard Protective Fungicides

Chemical	Rate of application equivalent of four cycles/treatment at	Mean sporulating lesions after treatment (per cent)
Copper oxychloride	3½ lb. in 1–2 gal./acre	28
Zineb	2 lb. in 1–2 gal./acre	17
3:1 mixture of copper oxychloride and 'Zineb'	3 lb. in 1–2 gal./acre	5
Untreated control	—	79

Gros Michel bananas in the Southern Cameroons. Here two experiments were attempted: site 1 on heavily infected mature banana plants; site 2 on a banana abandonment which was completely cut down then sprayed regularly during regrowth.

Application of chemicals to both sites was made at 14-day intervals with a Kiekins Deckar Motoblo machine applying 3½ lb. of copper oxychloride per acre in 1 gallon of water containing 4 per cent v/v amine/stearate formulant[5]. The experiments were commenced in November 1959 and disease recordings were made at two-weekly intervals on one site until the end of April. The technique of recording used was a modification of that suggested by Leach[2]. On site 1 a sample of ten parent plants and ten followers were recorded on each occasion. On site 2, however, two separate samples of 15 plants each were recorded during the final examination, and the results obtained in this manner are summarized in Fig. 1 and Table 2 respectively.

Table 2. Percentage of Leaves recorded in Each Disease Category on Treated and Untreated Plants developing on an Area that had previously been 'Cut Down' (Site 2)

Treatment	Disease category	Samples 1	Samples 2
3½ lb. copper oxychloride in 1 gal./acre*	Nil	56·4	56·4
	Light	15·4	21·4
	Medium	26·5	16·2
	Heavy	1·7	6·0
Untreated control	Nil	24·1	28·5
	Light	12·1	12·3
	Medium	19·3	23·1
	Heavy	44·5	36·1

* This site was sprayed four times between March 10, 1960, and May 1, 1960, when the final assessment was made.

Fig. 1 shows quite clearly that there is a very marked delay in the appearance of any significant difference between the control and the treated areas in mature plantations. This experiment was carried out during the dry season, and as such the incidence of disease was not at its maximum. D. Price (late of the Cameroons Development Corporation: personal communication) has confirmed that similar delays were obtained by the low-volume application of other copper fungicides in his trials which were commenced in July 1958. One possible explanation of this delayed effect is that the copper fungicides are acting primarily as anti-sporulating agents. If this interpretation is correct, then in order to obtain a more rapid control of the disease it would possibly be necessary to have much more cover than is normally given by a single aircraft application of chemical at 1–2 gal./acre, but provided a given fungicide deposit is sufficiently persistent then repeated applications should lead to a build-up of chemical on the older leaves equal to that given by any high-volume ground application. The upper leaves of the banana plant always appear free from disease, and mature lesions only appear on lower leaves in the plant. From a microscopic examination of approximately 6,000 lesions it appears that the 'black lesions' sporulate much more uniformly than any other type of lesion, although spores are also found on the older lesions. These black lesions are most commonly recorded about half-way down the mature plant, although again they may occasionally appear on very old or young leaves. Most of these observations suggest that the main conidia-producing areas of an infected plant are middle and lower leaves, and not until these leaves are adequately covered

with an anti-sporulating chemical will any marked effect of spraying from the air become apparent.

Eradicant oil applications from the air would certainly reduce the number of lesions developing to the 'black' stage, but does not prevent sporulation of any lesions that were already present[3,4], and thus a single oil application would not have any long-term effect in that the high inoculum potential present would remain. This suggests that provided climatic conditions remain conducive to the fungus, it would be necessary to apply oil to a given plant until the youngest leaf present during the first application had developed to complete maturity before the maximum effect on the incidence of disease was obtained.

Table 2 demonstrates that under the cutting-out routine, an almost immediate response to spraying was obtained, which suggests that under conditions of low inoculum potential brought about by the removal of the older infected leaves, the low-volume application of a copper fungicide also imparts some protection to the crop. This is the normally accepted explanation of the mode of action of these chemicals, but we would like to suggest that the anti-sporulation effect of such treatments as are mentioned in this report are possibly much more significant in determining field results than was previously realized. If this interpretation is correct, then the aerial application of protective and anti-sporulating fungicide could not be expected to show its maximum effects until (at least) 6–7 months after the commencement of that treatment. Furthermore, the deposits most resistant to weathering should have a marked advantage over less persistent materials.

We wish to express our thanks to the Directors of the Cameroons Development Corporation, United Fruit Co., and Fisons Pest Control, Ltd., for permission to publish these results. More particularly we would like to express our appreciation of the assistance given to us by various members of these organizations, including Drs. Forsyth, Fulton, Klein and Stover of the United Fruit Co., and Mr. Etherington and Mr. Price of the Cameroons Development Corporation.

[1] Guyot, H., and Cuille, J., *Fruit d'outre mer*, **10**, 101 (1955).
[2] Leach, R., *Banana Leaf Spot on the Gros Michel Variety of Jamaica* (Government Printers, Kingston, 1946).
[3] Calpouzos, L., Theis, R., Rivera, C. M., and Colberg, C., *Phytopath.*, **49**, 119 (1959).
[4] Klein, H. H., *Phytopath.*, **50**, 488 (1960).
[5] *Financial Times*, May 12, 1961.

AGE OF BED I, OLDUVAI GORGE, TANGANYIKA

By Dr. L. S. B. LEAKEY
Coryndon Museum, Nairobi
AND
Dr. J. F. EVERNDEN and Dr. G. H. CURTIS
Department of Geology, University of California, Berkeley

OLDUVAI GORGE is justly famous because of its unique geological sequence of Pleistocene deposits, which are exceedingly rich in fossil fauna, as well as a long sequence of stages of evolution of the earlier Stone Age cultures[1].

In the monograph[1] published in 1951 the view was expressed that although Bed I differed from Bed II in faunal content, both belonged to the lower part of the Middle Pleistocene. This view was revised by one of us in 1959 as a result of reviewing the fauna collected in the series of detailed excavations during the period 1952–59 inclusive.

Leakey claimed that it was now apparent that the time-interval between Bed I and Bed II was greater than had been previously supposed, and he reverted to the view which he had published in 1935[2] that Bed I was of Lower Pleistocene age. In view of the extraordinary wealth of fossil and cultural material in the Olduvai deposits, it was not surprising when in 1959 a most important fossil hominid skull—*Zinjanthropus boisei*—was found in Bed I, at site *FLK* I, in association with faunal remains and Stone Age culture material of the Olduwan culture[3]. In 1960, thanks to the Research Committee of the National Geographic Society, the Wenner Gren Foundation and the Wilkie Trust, very extensive further work was carried out, resulting in the discovery of pre-*Zinjanthropus*[4] fossil hominid material at a lower geological level in Bed I.

Fortunately, many of the deposits at Olduvai Gorge are pure volcanic deposits, containing no derived material, and are therefore usable for the potassium–argon dating method. A preliminary examination of deposits in the Gorge and collecting of material were carried out by two of us, Leakey and Evernden, in 1958 and additional material was collected at intervals and sent to Berkeley by Leakey in 1959 and 1960. In 1961, Curtis visited Olduvai Gorge with Leakey and Richard Leakey for further collecting and study. Although many of the samples collected have not yet been studied, we all three feel that a report on those specimens which have a direct bearing on the age of the fossil hominid remains in Bed I should be published, in view of the scientific importance of these early hominids.

The three sites which have yielded hominid remains in Bed I are: (*a*) *FLK* I, the site which yielded the skull of *Zinjanthropus boisei*; (*b*) *FLKNN* I, the site which yielded the remains of a 'child', representing a pre-*Zinjanthropus* hominid; and (*c*) *MK* I, which has not been referred to in the preliminary reports but which has yielded some teeth and parts of a skull of a hominid. All three sites have yielded an archaic fauna and tools of the Olduwan culture, and are regarded as of Lower Pleistocene (Villafranchian) age.

The Bed I samples which have been dated are as follows:

KA	412	Basal bed in the tuff and sediment sequence of Olduvai Gorge. Sample collected
	437	immediately west of third fault on south side of gorge at knife-edge descent. This horizon can be correlated with the series

Table 1

Top of Bed I						
KA 664	Biotite	1·39 gm.	6·96% K	$1·77 \times 10^{-11}$ mole A_{40}^{r}	93% A_{40}^{at}	$1·02 \times 10^{6}$ yr.
664*R*	Biotite	5·99	6·96	8·36	82	1·13
KA 861	Oligoclase	6·4	1·17	1·85	93	1·38
Bottom of Bed I (hominid-containing series of tuffs)						
KA 412	Anorthoclase	29·6	3·29	28·3	67	1·63
KA 437	Anorthoclase	6·68	3·06	6·62	70	1·74
KA 846	Anorthoclase	7·91	2·52	5·55	52	1·57
KA 847	Anorthoclase	5·90	3·18	6·16	70	1·85
KA 849	Anorthoclase	6·50	2·04	4·44	77	1·89
KA 850	Anorthoclase	8·17	3·94	10·20	41	1·78
KA 851	Anorthoclase	7·79	3·69	8·37	61	1·64

of tuffs in which hominid remains have been found by Leakey.

KA 846 Site *MK*, 18 in. below hominid remains. Buff coarse-grained tuff 13 in.–14 in. thick.

KA 847 Site *MK*, just above hominid layer. Light grey tuff, 20 ft. thick. Appears to be Pelean type ash. On every criterion, this must be considered an uncontaminated volcanic deposit.

KA 849 Site *FLK* I. 11-in. crystal tuff overlying *Zinjanthropus* layer.

KA 850 Site *FLKNN* I. 2-in. ash above very fine-grained 1-in. ash resting on pre-*Zinjanthropus* floor.

KA 851 Site *FLK* I. 12-in. tuff immediately under *Zinjanthropus* floor. Sample is rather weathered and soft with numerous Zinjtime root holes.

KA 664 Sample collected near top of Bed I, some distance from the hominid sites. At time 664*R* of collection, it was thought that this horizon was older than the hominid sites and it is so described in a paper submitted for publication by the INQUA Congress, Warsaw, 1961. However, more careful field work has established unequivocally that this horizon is definitely younger than any of the hominid sites.

KA 861 19-in. soft lapilli tuff, near top of Bed I and definitely younger than any of the hominid sites.

In the area of the three hominid sites, between 25 and 30 individual tuffs and tuffaceous beds can be distinguished in Bed I. At these sites these aggregate 39 ft.–43 ft. in thickness. Most tuffs are approximately a foot in thickness, but at the *MK* site the tuff immediately overlying the hominid floor is 129 in. In order of abundance, the tuffs fall into the following categories: anorthoclase-bearing vitric-crystal tuffs; anorthoclase-bearing crystal vitric tuffs; oligoclase bearing lapilli tuffs; and tuffaceous sandstones.

Within individual beds faint lines of stratification and evidence of size sorting may sometimes be seen. Some beds, however, show little or no sorting, vary tremendously in grain and lapilli size and exhibit characteristics of nuée ardente deposits. This is true of the tuff overlying the hominid remains at *MK* site. Some of the crystal vitric tuffs show marked crossbedding and have probably been re-worked. Most beds, however, appear to be primary ash falls.

Vitric shards in most of the tuffs have been more or less altered to clay, but the crystals appear fresh. Dark and strongly altered tops of some tuffs probably marked old soil zones. Numerous root casts below such zones strengthen this interpretation. Angular quartz and quartzite fragments are obvious in only a few of the tuffs. The nearest source of basement complex contaminants of this type is an inselberg of quartzite and gneiss approximately 1½ miles north of the hominid sites. More common visible contaminants are bone fragments. These are usually confined to thin strongly weathered layers; but in some cases, as in the tuff below *Zinjanthropus*, occur throughout the tuff.

Reck believed that the source of these tuffs was to the east, and the rapid thickening and coarsening of the strata in that direction is convincing proof that this is so.

At the *FLK* and *FLKNN* sites the hominid remains occur approximately 15 ft.–16 ft. above the basalt flow at the base of Bed I, while at the *MK* site, the hominid remains occur 9 ft. above the basalt. The samples dated, with the exception of *KA* 851 below the *Zinjanthropus boisei* floor which contained numerous bone fragments, showed no visible contaminants. In four of the six samples, the glass shards had been almost completely altered to clay. In one of these, *KA* 849, about 20 per cent of the glass shards were fresh, while in *KA* 847, from just above the hominid remains at *MK* site, most of the glass shards were fresh. Whether the more intense alteration of the four samples is the cause of their somewhat younger ages is not known, but it appears reasonable.

The run data and derived ages are given in Table 1.

The decay constants used in the computation of ages are $\lambda_\beta = 4·72 \times 10^{-10}$ yr.$^{-1}$, $\lambda_k = 0·584 \times 10^{-10}$ yr.$^{-1}$. The standard deviation of all of these runs is $0·1 \times 10$ yr. or less. This figure is, of course, simply a measure of the reproducibility of our results and does not attempt to deal with geological factors which might cause some scattering of the derived ages.

These samples were critically selected and examined and there seems to be no chance of their being contaminated by older eroded debris. It is to be noted that the hominid-site dates fail to fall in the proper relative order but all are between 1·6 and 1·9 million years. At the present time, the best estimate that we can make of the age of these sites is the average of the several ages, that is, 1·75 million years. This figure is in excellent agreement with *KA* 412, which is at the same stratigraphic level, but which was sampled a few miles from the hominid sites. The two samples from the top of Bed I give an average age of 1·23 million years, that is, 0·5 million years younger than the hominid sites. It may be of interest to note that we have obtained[5] an age of 360,000 years on a post-Chellean II tuff in Bed II, Olduvai. The conclusion is inescapable that Olduwan culture and Villafranchian fauna are synchronous in time and that both are approximately 1·75 million years old.

[1] Leakey, L. S. B., *Olduvai Gorge* (Camb. Univ. Press, 1951).
[2] Leakey, L. S. B., *Stone Age Races of Kenya* (Oxf. Univ. Press, 1935).
[3] Leakey, L. S. B., *Nature*, **184**, 491 (1959).
[4] Leakey, L. S. B., *Nature*, **189**, 649 (1961).
[5] Evernden, J. F., and Curtis, G. H., *Proc. INQUA Cong., Warsaw* (1961).

LETTERS TO THE EDITORS

SPACE SCIENCE

Interpretation of Cosmic Noise Measurements at 3·8 Mc./s. from Satellite 1960 η1

The results of measurements of cosmic noise at 3·8 Mc./s. from the satellite 1960 η1 have been reported by Molozzi, Franklin and Tyas[1]. A puzzling feature was the variation in field-strength at different locations. These results have now been analysed, on the assumptions that variations in signal strength are due to three causes: (*a*) the variation of radiation resistance of the antenna due to the ionosphere surrounding of the satellite; (*b*) the restriction in angular aperture of the satellite antennæ due to the ionosphere; and (*c*) the variation of cosmic noise power over the sky. The results indicate that these are the most important factors affecting the cosmic radio signals.

The variation of cosmic noise across the sky can be removed from the field-strength data by plotting noise-field contours along the orbit, using one of the sky maps such as that computed by Steiger and Warwick at 18 Mc./s. (ref. 2). The field-strengths can then be normalized to a standard value of the cosmic-noise field. In this communication the field-strength is normalized with respect to a region of the sky remote from the centre of the Galaxy, where the variation was small and the sky temperature low. Variation in antenna gain over the region is neglected.

The remaining variation in cosmic-noise levels is predominantly due to the effect of the ionosphere, neglecting for the moment the effect of the restriction in angular aperture. It can be shown that the radiation resistance of loop antennæ, such as those used in the experiment, varies as $\varepsilon^{3/2}$, ε being the dielectric constant of the medium. Therefore, as the antenna is immersed in ionosphere of increasing electron density, the resistance due to radiation will decrease, and hence the cosmic-noise power output from the receiver will also decrease.

The largest values of the electric field-strength are assumed to be representative of the noise field outside of the ionosphere, where the dielectric constant is unity. It follows that the ratio of the noise field-strength measured in the ionosphere to that outside the ionosphere, is proportional to $\varepsilon^{3/4}$, when absorption in the upper *F* region of the ionosphere is assumed to be negligible.

Since several modes, particularly the ordinary and extraordinary modes, can be propagated independently in the ionosphere, each one can contribute to the total noise-power at the satellite. The assumption was made that the energy was evenly divided between the two components above the ionosphere, that their signals added incoherently at the satellite and that the gyro-frequency was of the order of 1·4 Mc./s. at an altitude of 800 km.

Based on the hypotheses in the preceding two paragraphs, first estimates of the values of the dielectric constant and the electron density at the satellite were computed for each of the 22 observations reported by Molozzi *et al.* Since the largest values of the field-strength were observed over Woomera, these values were taken to represent the field-strength outside the ionosphere.

In order to arrive at more precise values of ε, it is necessary to allow for the angular aperture θ of the antenna, by a method of successive approximations. In the limiting case, $\sin\theta = \sqrt{\varepsilon}$.

Using the first value of ε calculated above, the angular aperture can be determined, and a more precise value computed for the noise-field received at the satellite, averaged over the region of the sky being viewed. Following the same procedure as before, a second approximation of the dielectric constant can be arrived at. The procedure can then be repeated and mean values of the electron density found by this means for the North American telemetry stations, Ottawa, Blossom Point and Fort Myers.

Date	Local time (approx.)	Mean height of satellite	Electron density
June 24–26, 1960	2200 hr.	870 km.	$9{\cdot}2 \times 10^4$ per c.c.
June 27, 1960	2200 hr.	855 km.	$10{\cdot}4 \times 10^4$ per c.c.
June 24–26, 1960	1400 hr.	1,050 km.	$7{\cdot}5 \times 10^4$ per c.c.

The difference in electron density between the measurements on June 24–26 and June 27, at the same local time, is approximately 10 per cent. Since a magnetic storm began with a sudden commencement at 0146 G.M.T. on June 27, it is surmised that the increase is due to the effects of the magnetic storm. The observations on June 27 were all made within 6 hr. of the beginning of the storm, during the initial positive phase of the variation of magnetic field.

The sky brightness temperature *T* computed from the results obtained over Woomera was found to be $8{\cdot}1 \times 10^6$ °K. in the vicinity of R.A. 5·5 hr., Dec. −30°, the region used for normalization of the values of field-strength.

A more detailed report will be given elsewhere.

J. H. Chapman
A. R. Molozzi

Defence Research Telecommunications Establishment,
Ottawa.

[1] Molozzi, A. R., Franklin, C. A., and Tyas, J. P. I., *Nature*, **190**, 616 (1961).
[2] Steiger, W. R., and Warwick, J. W., *J. Geophys. Res.*, **66**, 57 (1961).

ASTROPHYSICS

Apparent Recession of Nebulæ and the Nature of Radio Stars

In a recent communication[1m], a brief summary was given of the quantitative evidence in favour of the whirl theory of light and matter, according to which the whole physical universe is the manifestation of Maxwell's electromagnetic waves, wherein the fundamental particles of matter are represented as integral solutions of Maxwell's equations *in vacuo*, in cylindrical co-ordinates, extended from a low limit of the variable parameter of the integrant solution to infinity (the 'compound whirl'), while the photons are represented by the same kind of solution but with a

finite upper limit (the 'fragment'). The system of electromagnetic waves represented by the integrant solution was called the 'simple whirl'[1b,1j]. The energy of a fully realized compound whirl is finite, notwithstanding its infinite extension in space, and therefore the fundamental particles of matter are stable[1b,1j]. On the other hand, the total energy of a fully realized fragment would be infinite and therefore it can only be partially realized at the emission and then spread transversally asymptotically tending to its full realization and, at the same time, it must move in the axial direction asymptotically (though with great rapidity) tending to the theoretical 'velocity of light'[1j]. Thus the photon can be regarded as a fragment of a fundamental particle of matter. Its emission, however, does not affect the stability of the emitting fundamental particle, because the upper limit of the integral solution that represents it remains infinite after the emission and only the lower limit becomes slightly higher; its total energy remains finite. The quantum relationship at the emission is determined by Maxwell's electrodynamics[1j,f-l,a].

The spreading of the photon also implies a continuous increase of the wave-length λ, which very rapidly, though asymptotically, tends to proportionality to the distance from the source of emission according to the equation:

$$\frac{\Delta\lambda}{ct} = \frac{\sqrt{2\pi^3\chi_1}}{(q_0\ r_0)^4} \qquad (1)$$

(derived by simple transformation from formula (7·9), ref. 1*j*), where t is the time elapsed since the emission of the photon, c is the velocity of light, $\chi_1 = 0{\cdot}0575$ (*ibid.*, formula 8·5) is the average value of the above-mentioned dimensionless lower limit of integration of the integral solution representing the electron or the proton; $q_0 = mc/\hbar$ (where m is the mass of the emitting particle, $\hbar \equiv h/2\pi$ is the Planck constant); r_0 is the radius of the photon at the moment of emission.

This formula permits the determination of r_0 from the generally accepted results of observation. According to the data given by Sir Arthur Eddington[2]:

$$\frac{\Delta\lambda}{{}^0\lambda} = 5{\cdot}75 \times 10^{-34}ct \qquad (2)$$

where ${}^0\lambda$ is the wave-length of the photon at the emission.

It can be found from (1) and (2), on substituting the constants, that, for example, for ${}^0\lambda = 4 \times 10^{-5}$ cm. (violet light) $r_0 = 7{\cdot}6 \times 10^{-5}$ cm., whence $2\pi r_0/{}^0\lambda = 3{\cdot}8\pi$. From this it can be found (formula 9·8, ref. 1*b*) that the prevailing part (more than 93 per cent) of the energy of the emitting electron is contained within this radius and hence that the pattern of the emitting particle and of the emitted photon should not be substantially distorted at the emission.

Thus the shifting toward the red of the spectral lines of nebulæ, proportional to their distance from the point of observation, does not require for its explanation the hypothesis of the 'expanding universe' and hence it can scarcely justify the cosmic speculations generated from this hypothesis.

This is equally true in relation to similar phenomena in the radio stars. From the point of view of the whirl theory the radio stars should be regarded, at least for the time being, in the absence of more direct information about their nature, as consisting of atom and molecule-like formations of particle-like compound whirls of comparatively large dimensions ('macroparticles'), which should emit photons of correspondingly large dimensions ('macrophotons'). To evaluate the order of magnitude of macroparticles, we may assume that the scale relationship between the macrophotons and the emitting macroparticles is of the same order as between the photons and the emitting electrons. Taking again the violet light as an example, this ratio $h/(mc^0\lambda)$ is about 6×10^{-6}. The average order of wave-length of radio-astronomical signals, which we can regard as macrophotons, is about 10 cm. and hence the wave-length h/mc of macroparticles should be of the order of 5×10^{-4} cm. Thus, assuming that the Planck constant of the macroparticles is the same as of the fundamental particles of matter and of the photons, the mass of the macro-electron must be of the order of $3{\cdot}6 \times 10^{-33}$ gm. and that of the macroproton of the order of $6{\cdot}65 \times 10^{-30}$ gm.

It seems likely that macroparticles can, at least occasionally, be formed from the vortices (that is, revolving clouds) of particles of matter. As was pointed out on a number of occasions[1b,j,m], de Broglie undulations are implied in the solutions of Maxwell's equations representing the whirls. In an assembly of whirls (and hence also in an assembly of particles) they tend to become distributed in such a way that the pattern of de Broglie undulations by themselves should satisfy, or at least be near to, a solution of Maxwell's equations, superimposed upon the set of solutions representing the constituent whirls, so that the latter should form a fine structure of the former. No rigorous proof of this proposition was attempted, but it was applied tentatively to gravitation[1j,k,m], and it led to the correct determination of the period of rotation, and of the sidereal motion, and of the distance from the Sun, of Mercury, and of the period of rotation of Venus[1j,k,m].

It seems, therefore, likely that a system of de Broglie waves of protons and electrons revolving together in a vortex can occasionally emit a macroparticle the mass of which is very small in comparison with the mass of the vortex itself. The following consideration should increase the plausibility of this approach. The revolving particles in a vortex generally tend to acquire equal moments of momentum in relation to its axis. Hence their linear velocities tend to become inversely proportional, and the de Broglie wave-lengths tend to become proportional, to the distance from the axis, or, in other words, the angular wave-length of de Broglie waves tends to become constant. This answers the general description of electromagnetic whirl.

The macroparticles can form the atoms and molecules in the same way as the electrons and protons, and where their mass ratio equals the mass ratio between the proton and electron, they will form macroatoms and macromolecules geometrically similar to the atoms and molecules of matter.

N. S. Japolsky

Department of Electrical Engineering,
King's College,
London, W.C.2.

[1] Japolsky, N. S., *a*, *Phil. Mag.*, **19**, 934 (1931); *b*, **20**, 641 (1935); *c*, **30**, 641 (1935); *d*, **22**, 537 (1936); *e*, **22**, 1003 (1936); *f*, *Proc. Nat. Acad. Sci. India*, **9** (4), 213 (1939); **10** (1), 19 (1940); *g*, *Nature*, **137**, 582 (1936); *h*, **154**, 20 (1944); *i*, **159**, 580 (1947); *j*, *Pakistan J. Sci. Res.*, 111 (Oct. 1949); *k*, 1 (Jan. 1950); *l*, *Bull. Soc. Belge de Phys.* (Jan. 1958); *m*, *Nature*, **189**, 651 (1961).

[2] Eddington, Sir Arthur, *The Expanding Universe*, 67 (1933).

XUM

GEOPHYSICS

The Frequency of Meteorite Falls throughout the Ages

In a recent article[1] Prof. H. Pettersson describes his investigations of samples of deep-sea deposits in which were found metallic spherules, mostly less than 250μ in diameter, to which it is reasonable to ascribe a meteoric origin. These occurred in several geographical regions and in layers of various geological ages, and among the conclusions reached is the following: "In general, the number of spherules is greatest in the more recent sediments. Nevertheless, substantial numbers of spherules were found in layers of Tertiary age: this contradicts the assertion made by some authorities that no meteors fell on the Earth during that time". A reference identifies the "authorities" mentioned as the late Prof. F. A. Paneth, who, in a paper in *Vistas in Astronomy*[2], wrote: "The result of our discussion is that *glass meteorites fell on our Earth only during the late Tertiary and early Quaternary; that iron and stone meteorites did not fall before the late Quaternary; that centuries ago the fall of iron meteorites was a much more common phenomenon than to-day; and that even since 1800 meteorite falls seem to have become noticeably rarer*".

It is clear from Paneth's paper that he is discussing meteorites only, and the evidence which he gives for his statement can refer only to those bodies. He consistently emphasized the lack of evidence that meteors and meteorites have a common origin[3], and there is nothing in his conclusions that is inconsistent with Pettersson's findings. Pettersson uses the counts from his samples to generalize for the whole Earth, and concludes that the present rate of accretion is 1,000–5,000 tons per annum. Such a generalization from any sample of meteorite falls would clearly be quite invalid. It follows that Paneth's conclusions regarding the fall of meteorites in past times are unaffected by Pettersson's important observations.

In view of the recent revived interest in the analysis of meteorites[4], it might be timely to point out that Paneth's considerable collection of meteorites is now, by his wish, available for experimental study. The secretary of the "Paneth Meteorite Trust", who would welcome applications from qualified investigators, is Dr. H. Wänke, Max-Planck-Institut für Chemie, Mainz, Germany.

Herbert Dingle

104 Downs Court Road,
Purley,
Surrey.

[1] *Endeavour*, **19**, 142 (1960).
[2] *Vistas in Astronomy*, **2**, 1686 (1956).
[3] See, for example, *The Origin of Meteorites* (Halley Lecture, 1940, Clarendon Press, Oxford).
[4] See, for example, Bernal, J. D., *Nature*, **190**, 129 (1961).

Murray's and Renard's interpretation of the "cosmic spherules" found in deep-sea deposits[1] that they are of cosmic origin, and were probably derived from iron meteors heated to superficial melting during flight through the atmosphere, seems to be generally accepted at present. Having myself studied hundreds of such spherules, extracted from deep-sea cores taken by the Swedish *Albatross* Expedition (1947–48), I have found Murray's description of their external structure confirmed. In addition, analyses of the composition of individual spherules by the Castaign microanalyser[2] has confirmed that they contain, besides iron, also nickel and cobalt, which supports their extra-terrestrial origin as well as Murray's hypothesis that they are derived from meteors of the nickel-iron type.

The resemblance between their surfaces and that of the iron meteorites seems to support the view that they may be derived from meteorites as well as from meteors which have not reached the surface of the Earth. The presence of cosmic spherules in deposits of Tertiary age (recently confirmed by Germans investigating the "Buntsandstein" in north-west Germany) certainly supports the view that they indicate meteoric activity long before the Quaternary.

Whether the origin of meteorites and ordinary meteors is different seems to be an open question. Whipple assumes most meteors to be of cometary origin, whereas meteorites are usually assumed to be derived from planetary fragments like the asteroids. It seems to me that the origin of the meteorites and, still more, of the tektites is still under debate, whereas the origin of the cosmic spherules is that announced by Murray.

My calculation that the accretion of cosmic spherules to the Earth is 1,000–5,000 tons annually is, of course, due to extrapolation from the counts made by myself and my co-workers in a limited number of cores. I hope to obtain a more dependable figure from the much longer cores to be raised by the Moho-Project.

Hans Pettersson

Oceanografiska Institutet,
Göteborg.

[1] *Proc. Roy. Soc. Edin.*, **9**, 258 (1876–77). *Challenger Rep.*, **4**, *Deep Sea Deposits*, 327.
[2] Castaign, R., and Fredriksson, K., *Geochim. Cosmochim. Acta*, **4**, 114 (1958).

RADIOPHYSICS

Scintillations and the Latitude Distribution of Ionospheric Irregularities

Kent[1] has reported a series of observations on the 40 Mc./s. transmissions from *Sputnik* 1 made at Cambridge during October 1957. He found that the transmissions always underwent rapid fading (scintillation) when the satellite was north of Cambridge but only occasionally when south of Cambridge. He concluded that the scintillations were due to ionospheric irregularities which are north of the observing station but not south of it. Further, since the southward extent of the scintillation region was greater for satellite passes to the west of Cambridge than to the east, he postulated that the density of the irregularities is controlled by the magnetic rather than geographic latitude (Cambridge's declination is about $-9°$). Mawdsley[2] objected to Kent's seemingly arbitrary assumption that the irregularities do not occur south of the observer, on the grounds that radio star scintillations are observed at lower latitudes than that of Cambridge. He suggested an alternative interpretation of Kent's results in terms of preferential forward scattering by the irregularities, assumed to be field aligned, when the line of sight from the observer to the source is normal to the field lines. Afterwards, Bain[3] and Frihagen and Tröin[4], working at Slough and Kjeller respectively, observed scintillations when the satellite under observation was south of the observing station. However, they both found that the scintillation activity increased for geomagnetic

latitudes greater than that of Cambridge, that is, 55°. This led Frihagen and Tröin to support Kent's interpretation. It is the purpose of this communication to point out the existence of further pieces of evidence which support Kent's point of view rather than Mawdsley's.

It has long been known that the scintillation of radio stars correlates strongly with the occurrence of spread-*F* (for example, Booker[5]), and recent work[6,7] has shown this correlation applies to scintillation of satellite signals also. The distribution of scintillation activity with latitude may therefore be expected to be similar to that of spread-*F*. Singleton[8] has shown that the distribution of spread-*F* depends on geomagnetic latitude and, further, that between 60° and 70° the percentage occurrence rises from about 10–80 per cent or more. Simple calculation shows that the north–south tangent to the Earth's surface at an observing point cuts the 300-km. level at points the latitudes of which differ from that of the observing point by ±17°. For Cambridge (geom. lat. 55°) the range of geomagnetic latitudes over which the 300-km. level is observable is thus 38° N.–72° N. For the 400-km. level this range is 35° N.–75° N. It is clear, therefore, that if the irregularities responsible for the spread-*F* and scintillations are in the 300–400 km. region, as is believed to be the case, the observed marked increase in scintillation activity when the satellite is north of Cambridge is to be expected. This explanation does not preclude the possibility of observing scintillations at geomagnetic latitudes south of 55° since the incidence of spread-*F* nowhere falls to zero, and indeed, since spread-*F* activity increases again at the geomagnetic equator, an increase in scintillation activity would be expected here. This is observed[9].

The departure of the zenith angle dependence of the annual mean radio star scintillation index from the simple secant law, as pointed out by Hewish[10] in Cambridge and Little and Maxwell[11] in Manchester, can possibly be explained in a similar way. This secant law, which takes into account the variation of layer thickness with changing zenith angle, tacitly assumes a constant distribution of irregularities over the region of observation. The Cambridge and Manchester data are obtained mainly from observation of Cygnus and Cassiopeia, the lower culmination of each of which is near the northern horizon of these stations. Thus the larger the zenith angle, the further will the point of intersection of the radiation with the F_2 layer penetrate into the region of incidence of high irregularity to the north, and the larger will be the departure from the secant law. It can be shown that the latitude of the point which is at a height of 300 km. on a ray making a zenith angle of 60° at the observer in the meridian plane differs from the latitude of the observer by 4°. Since Cambridge and Manchester have geomagnetic latitudes of about 55° and 56° respectively and the increase in irregularity activity commences at geomagnetic latitude 60°, the departure from the secant law would be expected to commence at zenith angles of about 60°, as is observed.

An increase in scintillation activity with zenith angle has also been observed at middle latitudes[12], where the latitude distribution of irregularities is flat. However, the crude method of analysis used in this case makes it impossible to draw any conclusion as to whether the secant law can explain the variation or not.

Mawdsley's contention that the most favourable condition for forward scattering by field-aligned irregularities is when the incident radiation is normal to the field lines is contrary to experiment. Singleton, Lynch and Thomas[13], working with a field of view (geom. lat. 15° S.–55° S.) over which the incidence of spread-*F* is substantially flat, have shown that an enhancement in scintillation activity is experienced for propagation along the field lines, there being no enhancement for propagation normal to the field lines.

It would appear, therefore, that Kent's original interpretation of his results is in accord with the spread-*F* findings and that the scattering mechanism envisaged in Mawdsley's alternative explanation is not borne out by experiment. As a result it is asserted that Kent's explanations must be accepted.

D. G. Singleton

Physics Department,
University of Queensland.

[1] Kent, G. S., *J. Atmos. Terr. Phys.*, **16**, 10 (1959).
[2] Mawdsley, J., *J. Atmos. Terr. Phys.*, **18**, 344 (1960).
[3] Bain, W. C., *Nature*, **189**, 129 (1961).
[4] Frihagen, J., and Tröin, J., *J. Atmos. Terr. Phys.*, **20**, 215 (1961).
[5] Booker, H. G., *Proc. Inst. Radio Eng.*, **46**, 6363 (1958).
[6] Yeh, K. C., and Swenson, G. W., *J. Geophys. Res.*, **64**, 2281 (1959).
[7] de Mendonca, F., Villard, O. G., and Garriot, O. K., *Proc. Inst. Radio Eng.*, **48**, 2028 (1960).
[8] Singleton, D. G., *J. Geophys. Res.*, **65**, 3615 (1960).
[9] Koster, J. R., *J. Atmos. Terr. Phys.*, **12**, 100 (1958).
[10] Hewish, A., *Proc. Roy. Soc.*, A, **214**, 494 (1952).
[11] Little, C. G., and Maxwell, A., *Phil. Mag.*, **42**, 267 (1951).
[12] Bolton, J. G., Slee, O. B., and Stanley, G. G., *Austral. J. Phys.*, **6**, 434 (1958).
[13] Singleton, D. G., Lynch, G. J. E., and Thomas, J. A., *Nature*, **189**, 30 (1961).

PHYSICS

Pulse-shortening in Electron Linear Accelerators

Kelliher and Beadle[1] have published some preliminary observations of the phenomenon of pulse shortening in electron linear accelerators.

Since the problem was first noted[2] investigations have also been in progress in our laboratories. It would now appear that a satisfactory theory has been evolved and experimentally verified as a result of which various methods of avoiding the problem can be devised. Kelliher and Beadle suggested that the effect could be due either to spurious radio-frequency modes or to a 'field effect' associated with power level. It has in the past been difficult to see how a spurious mode could attain sufficient power-level to produce the very large forces required to affect a high-energy beam.

This difficulty has now been resolved by the realization of a significant difference between the action of forward and backward waves. The expression for the tranverse forces associated with a forward travelling-wave acting on a beam contains a term of the form $(1/\beta - \dot{z}/c)$, where β is the ratio of the phase velocity to light velocity, and $\dot{z}$ and c represent beam and light velocity respectively. For a forward wave this term approaches zero as β and $\dot{z}/c$ approach unity, indicating a balance of transverse electric and magnetic forces.

Considering a backward wave, however, it can be seen that in order that energy be transmitted in a direction opposed to the phase velocity the sign of the Poynting vector must be reversed, that is, the *H* vector must show a phase reversal in its relationship to the *E* vector as compared with the situation in a forward wave. From this, it follows that the sign in the expression given above will be changed and

the electric and magnetic terms will now add and thus any disruptive forces will, in a backward wave, be very much higher than in an otherwise similar forward mode. Consideration of the higher modes which exist in a corrugated wave-guide structure does reveal the existence of backward waves. It can thus easily be seen that there is, in the normal form of linear accelerator containing long uniform sections of corrugated wave-guide, the possibility of action as a backward wave oscillator and, even if only modest power be produced, transverse forces could be large.

An experimental investigation of possible modes in a corrugated wave-guide structure having $a/\lambda = 0{\cdot}11$ and for which the normal E_{01} mode has $\beta = 1$ at 2,998 Mc./s. has been carried out by I. White of this laboratory. The measurements show that there is (among others) an E_{11} mode which is a backward wave and that this wave has a phase velocity equal to light velocity at 4,436 Mc./s., under which conditions its group velocity is 0·038 c. Perturbation measurements of the series impedance (E^2/P) confirm that mode powers of the order of kW. would cause transverse forces sufficient to deflect a high-energy beam. One might therefore expect that a uniform accelerator section of $1\frac{1}{2}$ m. length of this guide fed by a bunched beam travelling at near light velocity would oscillate at a frequency of 4,432–4,433 Mc./s., the slight shift in frequency being necessary to allow for the phase-change between beam and wave from end to end of the section. A bunched beam of energy 8 MeV. and indeed 16 MeV. has been injected into such a section of accelerator and pulse-shortening effects were exhibited both when energized at a power-level of 5 MW. from an *S*-band klystron and when completely unenergized. Measurements were made at the input end of this section and power was detected at a frequency of 4,433 Mc./s. Examination of the variation of this power with time indicated a rapid increase at the end of the shortened electron pulse. When beam current was reduced until pulse-shortening no longer took place, no power at 4,433 Mc./s. could be detected. There seems no doubt, therefore, that pulse-shortening in this case was associated with backward wave oscillation in the E_{11} mode. Means were taken as mentioned below to reduce the possibility of E_{11} wave build-up and no pulse-shortening could then be detected.

These findings confirm the suggestion that the E_{11} mode is responsible made in the paper by Nygard and Post[3], although they did refer to an E_{110} mode of oscillation. The significance of the 0 is a little obscure as this would with normal cavity nomenclature imply a phase velocity of infinity, that is, that the wave was operating at cut-off. With the type of oscillation we are considering it is, of course, not necessary that the guide should act as a resonant cavity. The E_{11} mode near $\beta = 1$ is such that to a first approximation the fields in the axial region may be regarded as those of a plane wave with, of course, the significant difference that the E and H vectors are phased so as to give the backward group velocity, and the oscillatory motion of the beam will, therefore, be purely transverse. For this reason the longitudinal bunching of the beam at the E_{01} frequency has little or no influence on the build-up of oscillation. The theory has been carried to the extent of fitting known facts qualitatively and rough quantitative agreement has also been obtained. It is hoped that a complete theory will be published in the near future.

Having determined the causes of trouble it is relatively simple to devise means for avoiding them. On the above theory it is quite clear that the constant field guide suggested by Kelliher and Beadle with its essential non-uniformity would avoid the difficulty of pulse shortening. Similarly, arrangements using combinations of shorter uniform sections of different a/λ as used in some of our own accelerators would again avoid the difficulty. More elegant methods of avoidance are possible; for example, new accelerating structures may be used which are incapable of propagating the mode at velocities near that of light, such as that mentioned by Nygard and Post (possibly having $2\pi/3$ phase change per corrugation). Techniques using short sections which cause phase shift between the E_{11} mode and the beam have been used in our own laboratories. It is perhaps worth noting, however, that there are other higher modes which may cause similar troubles although it seems probable that higher starting currents for the oscillation would be required. Although, at present, difficulties seem to have been removed, similar troubles may occur due to these higher modes if and when beam currents are pushed an order of magnitude higher. In this connexion, it is perhaps significant that in the experiment with the accelerator section, mentioned above, a small amount of power has been found to exist under pulse-shortening conditions, at other frequencies, particularly at approximately 7,970 Mc./s. This mode has not yet definitely been identified, but may possibly be the E_{21}. Although it has been reported that pulse-shortening has not been observed on *L*-band accelerators, it is quite clear that such high mode problems could exist and it is probable that they would be found if the beam current were increased sufficiently.

As regards performance of *S*-band accelerators, we have in our laboratories a single-section accelerator operating from a 5-MW. klystron and have achieved beam current in excess of 1 amp. at 2 μsec. pulse-length and at an energy of 4 MeV., that is, almost complete radio frequency power absorption without any signs of pulse-shortening.

M. C. Crowley-Milling
T. R. Jarvis
C. W. Miller
G. Saxon

Research Department,
Associated Electrical Industries
(Manchester), Ltd.,
Trafford Park,
Manchester, 17.

[1] Kelliher, M. G., and Beadle, R., *Nature*, **187**, 1099 (1960).
[2] Boag, J. W., and Miller, C. W., *Proc. Second Conf. Geneva*, Paper P/62 U.K. (1958).
[3] Nygard, J. C., and Post, R. F., *Nuclear Instrum. and Methods*, 2, No. 1, 126 (1961).

Displacement Potentials and Bending of Rod-like Polyelectrolytes

In 1954, Jensen *et al.*[1,2] discovered an electromechanical property of solutions of potassium hyaluronate. They named the effect 'flow-potential', but agreed later on to change the name into 'displacement-potential'. The original measurements were performed with techniques including amplification. Experiments in 1959 by us[3] (C. E. Jensen died in November 1959) showed that the classical potentiometric technique without amplification was quite sufficient to measure the potentials. The new measurements corroborated the original findings. Beyond this Jensen and Vilstrup discovered that the poten-

tials were independent of the distance between the electrodes[4].

It seems that the observed phenomena can be derived from the properties of a crude model of the hyaluronate molecule: the molecule may be described as a long cylinder near the core of which a great number, for example 1,000, of negative elementary charges are distributed evenly. The core is surrounded by a sheath of positive electricity evenly distributed on a cylindrical surface. This assumption is based on the fact that the potassium ions are prevented by elastic and electric forces from moving in directions perpendicular to the surface while they are (nearly) free to move in the other two directions. We now inquire what will happen if we bend the cylinder. From the geometry of the toroid, it is known that a cylinder can be bent without any change in surface. What happens is that part of the surface is moved from the inside to the outside. Since the radii of curvature in question are large compared with the diameter of the cylinder, we must assume that the bending cannot cause significant changes in the even distribution on the surface. Therefore, the electrical effect of the bending will be the following: (*a*) if a number of molecules are bent simultaneously the same way, a certain, not negligibly small, amount of electricity will be displaced from the inside to the outside of the molecules under bending; (*b*) when the bending has ceased and the bending stress has disappeared, the bent molecules will tend to unbend, that is, they will tend to chase electricity in the opposite direction, against their centres of curvature. In other words, the entire solution, after bending, is in a state of strain which manifests itself as an electromotive force, tending to chase electricity in the direction from the molecules to their centres of curvature.

A typical experiment is performed as follows: a glass capillary is partly filled with a dilute solution of potassium hyaluronate, for example, with a 1 per cent solution. Two electrodes enter the solution from the sides of the tube. The discoverers of the effect observed two phenomena: when the solution is displaced from left to right relatively to the capillary, the measuring instrument shows that the right electrode is negative during the displacement and when the displacement has ceased its sign is reversed. Thus, the right electrode becomes positive and remains so, as long as the solution remains in the displaced position, or rather, the potential decays slowly, by some 10 per cent in the course of a day.

It is evident that, on account of the shearing forces existing in the solution during its displacement in the capillary, the long molecules must be bent so that most of them will have their insides pointing in the direction of the movement of the liquid. It will, therefore, be seen that predictions based on the model described here are in complete qualitative agreement with the observations. This agreement is corroborated by a third observation: when the capillary is emptied after an experiment and refilled with water without rinsing it, a displacement potential about ten times as large as before is observed[3]. In these circumstances, most of the molecules adhere to the glass and their bendings will be more acute, which must result in an increase of potential. Filling the capillary (internal diameter about 0·7 mm.) with thin glass rods has a similar effect[5].

The order of magnitude of the potentials is 5–10 mV. for experiments of the ordinary type, and for those of the kind described about 100 mV. Preliminary estimates based on the model in question yield results agreeing with these values. It follows from the estimate that the potential must be inversely proportional to the length of radius of curvature.

More detailed papers on the theory of the effect and its applications to the mechanism of hearing will be published elsewhere.

J. A. Christiansen
C. E. Jensen
Th. Vilstrup

Sundvænget 9,
Hellerup, Denmark.

[1] Jensen, C. E., Koefoed, J., and Vilstrup, Th., *Nature*, **174**, 1101 (1954).
[2] Vilstrup, Th., Jensen, C. E., and Koefoed, J., *Ann. Otol., Rhinol. Laryngol.*, **64**, 406 (1955).
[3] Jensen, C. E., Christiansen, J. A., and Vilstrup, Th., *Symposium über Makromoleküle in Wiesbaden, Kurzmitteilungen*, **5**, A, 11 (1959).
[4] Jensen, C. E., Christiansen, J. A., and Vilstrup, Th. (Lecture delivered by Th. Vilstrup at Colloquium Oto-Laryngologicum in Padua, 1960).
[5] Carlsen, F., Dansgaard, W., and Jensen, C. E., *Acta Chem. Scand.*, **13**, 1851 (1959).

Electrical Effects observed in Polycrystalline Boron

The conductivity of vacuum-fused polycrystalline specimens of boron is observed to increase in a magnetic field instead of decreasing as expected theoretically. Different samples exhibit the effect to different extents, but all show saturation at a field-strength of about 3,000 gauss. Increases of up to 1 part in 10^3 at saturation have been observed.

Preliminary measurements indicate that the effect persists at low temperatures but decreases above room temperature, falling below the noise-level at about 100° C. in the most sensitive specimen.

The effect shows no temperature hysteresis and is unaffected by changing the ambient atmosphere from air to argon. It is independent of the orientation of the magnetic field relative to the current vector.

All specimens examined were *p*-type by thermoelectric measurement, and contained a variety of traps as evidenced by the long time-constants observed in other measurements. The 'magnetoconductance' effect itself, however, could be measured at frequencies up to 10 kc./s., the limit set by the measuring system used. No reliable zero-frequency measurements have been made owing to the difficulty of maintaining the boron temperature constant to 0·01° C., rendered necessary by the strong dependence of conductivity on temperature.

Samples of polycrystalline boron also exhibit a 'charge storage' effect. To show this, current is passed through a piece of boron for a few seconds and then the source of current is replaced with a current meter. A charge passes through the meter in the same direction as would result from charging and discharging a capacitor in the same way.

Typically, a 100-V. battery is used to charge a 2-mm. thick slice cut from a 6-mm. diameter rod of boron and 10^{-8} coulomb flows through the meter. The discharge curve can be resolved approximately into two decreasing exponentials, with time-constants of 6 sec. and 180 sec.

In a further experiment, the boron is lowered into liquid nitrogen with the battery still connected, thereby 'freezing in' the charge. On substituting the meter for the battery and allowing the boron to warm up, charge is released at certain temperatures which are presumably related to the depths of specific

kinds of traps in the boron. The experiment is thus analogous to the production of a 'glow curve' in the examination of a luminescent material.

Some of the boron used in these investigations was prepared in this laboratory by an electron beam zone melting process and the remainder purchased as zone-refined material prepared by the Eagle-Picher Research Laboratories, Miami, Oklahoma.

Electrical connexion to the boron was through baked-on silver paste contacts which were substantially ohmic.

The observations described differ from, but may be related to, the 'magnetic pulse' and 'colour memory' effects studied at the U.S. Army Signal Research and Development Laboratory, Fort Monmouth, New Jersey[1].

C. H. Carmichael
M. Dore

Signals Research and Development Establishment,
Ministry of Aviation,
Somerford,
Christchurch, Hants.

[1] Gaulé, G. K., Breslin, J. T., Pastore, J. R., and Shuttleworth, R. A., *Boron*, edit. by Kohn, J. A., Nye, W. F., and Gaulé, G. K., 159 (Plenum Press, Inc., New York, 1960); and private communication to P. H. R. Scholefield of this laboratory.

GEOLOGY

Age of the Orogeny and Granites in South-West England

Mr. Butcher's valuable contribution[1] to this puzzling problem raises a number of points which I would like to answer.

First, there is no evidence that the deformation of the pre-Permian rocks of south-west England was delayed until Stephanian or post-Stephanian times. On the other hand, there is some evidence[2] that metamorphosed, schalstein and spilite areas (and perhaps even the Delabole Slates) were being eroded somewhere south of Wales in Pennant Series (that is, Morganian) times. Hints of early Morganian instability occur in the Bristol Coalfield and in the south-eastern portion of the South Wales Coalfield. Dr. Kelling has recently been studying Pennant rocks in South Wales and is finding that over considerable southern and central areas of that coalfield a mainly southerly source (not too far away either) and a parent mass of rocks like those of Devon and Cornwall are indicated.

Secondly, similarity of fold system[3] does not necessarily prove contemporaneity of deformation. The folded and thrust south-west England sediments (the age of which has not been proved beyond about the Modiolaris Zone) are separated from the Somerset area by the powerful Cannington Park thrust-belt (which could have brought into juxtaposition rocks deformed at different times of the Carboniferous).

Thirdly, no one has ever suggested that the granites were being eroded during Pennant Sandstone (or even later Carboniferous) times. I agree with Mr. Butcher that these granites, when intruded, would be deeply buried. I also agree that "at the present level of erosion, the youngest sediments so far recognized by fossils are referable to the Upper Gastrioceras Zone[1]". There could still, however, have been an appreciable thickness of later Ammanian (for example, Similis-Pulchra Zone) deposits above this "present level of erosion" when the granites were intruded. In other coalfields of England and Wales, the thickness of the Ammanian succession above the Amman Marine Band is at least equal to (and in some cases almost double) that of the underlying Coal Measures. This thickness in the Somerset Coalfield (the one nearest Devon) is about 2,000 ft. If the south-west England area was experiencing a renewal of thick greywacke-type deposition in later Ammanian times, then that higher succession could have attained a thickness of many thousands of feet. Prentice (ref. 4, p. 408) has suggested that quite thick greywackes could have been deposited in a relatively short space of time. The granites could then have been intruded during early Morganian times and yet be buried under a considerable thickness of earlier Coal Measure rocks.

I would (with caution) suggest another possibility to account for the depth of burial in Morganian times of the granites. Great thrusts occur in the Armorican fold belt of Cornwall and south Devon. Such thrusts may have carried masses of Devonian, or older, rocks (removed by later erosion) well north over areas of deformed Culm. There could then have been thrust-masses of pre-Carboniferous rocks on top of the Culm, (*a*) when the granites were being intruded and (*b*) when the Pennant Series was being deposited farther north. Such an interpretation might even explain the presence of Devonian limestone pebbles (of southern aspect) in the New Red Sandstone near North Tawton, Devonshire[5]. The high percentage of Devonian fragments in the Permo-Triassic breccias of south-west England[6] might also be explained in this way.

Lastly, while derived Dartmoor granite is unknown in these breccias, a considerable percentage includes porphyries which could have been connected with the granite intrusions[6].

I therefore maintain that the case for a very late Carboniferous orogeny in south-west England, and also for a Permian granitic injection, has not been proved. On the contrary, available evidence supports an earlier (probably early Morganian) age for the main deformation of the Devonian and Culm rocks of Devon and Cornwall. The granite intrusions probably accompanied this deformation.

T. R. Owen

Geology Department,
University College of Swansea.

[1] Butcher, N. E., *Nature*, **190**, 253 (1961).
[2] Heard, A., *Geol. Mag.*, **59**, 83 (1922).
[3] Kulp, J. L., Long, L. E., Giffin, C. E., Mills, A. A., Lambert, R. St. J., Giletti, B. J., and Webster, R. K., *Nature*, **185**, 495 (1960).
[4] Prentice, J. E., *Quart. J. Geol. Soc. Lond.*, **116**, 397 (1960).
[5] Hutchins, P. F., *Geol. Mag.*, **95**, 119 (1958).
[6] Wells, A. K., and Kirkaldy, J. F., *Outline of Historical Geology* (London, 1958).

I feel that the evidence cited by Mr. T. R. Owen does not necessarily support an Upper Westphalian (Morganian) age for the orogeny and granites. An intra-Westphalian orogeny could only be satisfactorily demonstrated by the discovery of younger unconformable Carboniferous sediments, as, for example, in northern Spain[1]. Such post-orogenic sediments are not known in Devon and Cornwall. However, our knowledge of this region is still very meagre, and it may be that they will yet be found. Meanwhile, it seems to me preferable on the present evidence to correlate the main orogeny in Devon and Cornwall with that in adjacent regions to the north. In this, I agree with Kulp *et al.*[2] and Lambert and Mills[3]; but also with Mr. Owen that the case has not been proved.

I imagine that a major orogeny ending with a granite batholith and associated mineralization, such as we now see exposed for more than 100 miles in south-west England, would require a considerable time (perhaps 20 million years) for its development. The whole of the Carboniferous period is thought to be 65 (ref. 4) or 80 (ref. 5) million years. Therefore, with pre-orogenic sedimentation known to be still going on in Lower Westphalian times, I suspect that the granites (and there is more than one time of granite intrusion) could not have been emplaced in the Upper Westphalian. It is of interest that the most recent age determinations of the Dartmoor granites[6] give a slightly *younger* age compared with previous values. The new date of 265 ± 5 million years falls nicely within the Permian period on Kulp's time-scale[4].

N. E. Butcher

Geology Department,
University of Reading.

[1] Wagner, R. H., *Estudios Geologicos*, **16**, 55 (1960).
[2] Kulp, J. L., Long, L. E., Giffin, C. E., Mills, A. A., Lambert, R. St. J., Giletti, B. J., and Webster, R. K., *Nature*, **185**, 495 (1960).
[3] Lambert, R. St. J., and Mills, A. A., *Ann. N.Y. Acad. Sci.*, **91**, 378 (1961).
[4] Kulp, J. L., *Science*, **133**, 1105 (1961).
[5] Holmes, A., *Trans. Edin. Geol. Soc.*, **17**, 183 (1959).
[6] Dodson, M. H., Miller, J. A., and York, D., *Nature*, **190**, 800 (1961).

CHEMISTRY

Evaporated Carbon Films of Large Area for Adsorption Studies by Electron Bombardment

The use of evaporated carbon films for adsorption studies is desirable in view of the difficulty of outgassing carbon powder. By analogy with the comparative properties of metal surfaces produced by evaporation and by reduction of powders, it seems likely that evaporated carbon should be significantly cleaner and more reproducible.

Following common practice with metal films, Sykes and Thomas[1] used a carbon filament mounted on tungsten leads with 'Aquadag'. The extent of evaporation was limited to a few per cent of the total carbon by electrical failure due to 'hot spots'. As a result the film thicknesses were generally of the order of 500 Å., corresponding to weights of the order of 2 mgm. Krypton adsorption isotherms showed that the residual filament had an area several times greater than that of the film, hence it was necessary to remove the remaining filament through a constriction and to seal off. This is inconvenient, and an undesirable feature if cleanliness is to be maintained.

The new technique avoids this difficulty by allowing almost 100 per cent evaporation of the carbon used. A second improvement is that only a light electrical contact to the carbon is required so that difficulties of mounting the carbon are reduced, 'Aquadag' can be dispensed with, and the outgassing of the supports can be made negligible. The film vessel is shown in Fig. 1. A small cylindrical piece of pure carbon (*A*) is supported on a tungsten rod (*B*) so that its tip is within a one-turn tungsten coil cathode (*C*). The cathode is heated to a controlled temperature by means of a 'Variac' and step-down transformer, and a positive potential of 500–2,000 V. is used to accelerate the emitted electrons to the carbon anode. The tungsten support is shielded from bombardment at

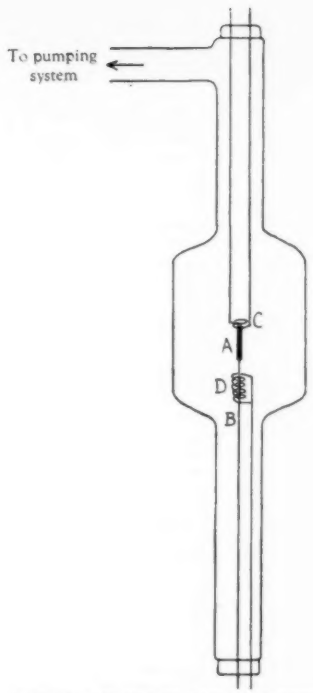


Fig. 1. Diagram of vessel used for carbon evaporation

high anode potentials by a repelling guard electrode (*D*). Depending on the size of the carbon anode a power of about 40 W. is sufficient for evaporation, after prolonged outgassing with a slightly reduced power. As a consequence of the small size of the anode and its almost complete evaporation the power dissipated within the silica film vessel during evaporation is much less than before while film thicknesses are considerably greater. Films as heavy as 50 mgm. have been obtained, but there is a tendency for such thick films to peel from the walls of the vessel.

A krypton isotherm, obtained at −196° C., on a film weighing 11·8 mgm. deposited without cooling the silica bulb, is shown in Fig. 2 with the corresponding B.E.T. plot. An almost equally straight line is

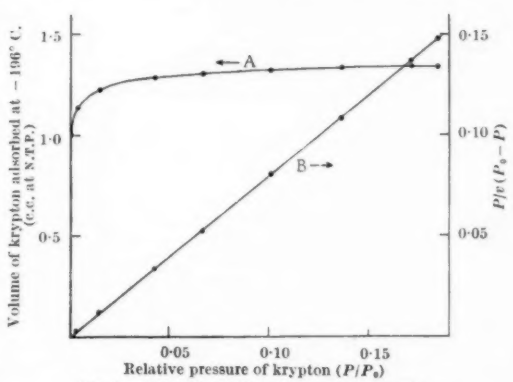


Fig. 2. Absorption of krypton on a carbon film

obtained from a Langmuir plot, as reported for graphite[2]. The B.E.T. monolayer volume is 1·24 c.c. at N.T.P., Taking 20 Å.^{2} for the effective cross-sectional area of a krypton atom, the film area is 6·67 m.^{2} or 565 m.^{2}gm.$^{-1}$. Films of this area, which is such that contamination hazards are much less severe than before, are readily prepared by the new method. The specific surface area is some ten times greater than that of the thinner films prepared by Sykes and Thomas, and is only slightly affected by baking at 550° C. A study of the relation of film area to film thickness is under way.

A. E. Bell
J. Pritchard
K. W. Sykes

Chemistry Department,
Queen Mary College
(University of London),
Mile End Road,
London, E.1.

[1] Sykes, K. W., and Thomas, J. M., *Proc. Fourth Carbon Conf.*, 29 (Pergamon Press, 1960).
[2] Ross, S., and Winkler, W., *J. Coll. Sci.*, **10**, 330 (1955).

The Marangoni Effect and Liquid/Liquid Coalescence

The rate of coalescence of liquid drops (phase 1) suspended in an immiscible liquid (phase 2) has been shown to increase when a third component, soluble in both phases, diffuses out of the drop[1–5], and vice versa. This behaviour has been attributed to the Marangoni effect[6], whereby interfacial flow is established by gradients in interfacial tension causing an increase in one case, and a decrease in the other, of the rate of thinning of the phase-2 film separating the coalescing interfaces.

We have studied the buoyant rise of a drop of water (phase 1) through cinnamaldehyde (phase 2) to a flat phase 1/phase 2 interface, using an interferometric method[8] to measure the rate of thinning $-(dh/dt)$ of the intervening phase-2 film. For drops 650μ diam., the thinning followed the relation[3]:

$$-\frac{dh}{dt} = kh^3$$

Table 1 lists experimental values of k and the corresponding values of the mean rest time $\bar{\tau}$ (ref. 3), which offer strong support of the foregoing hypothesis. Similar, but less complete, results were obtained from the system water/diphenyl sulphide.

Table 1

	$\bar{\tau}$ sec.	10^6k cm.$^{-2}$ sec.$^{-1}$
1 per cent acetone in phase 1	5·3	$\geqslant 3$
Pure system	8·1	3·0
1 per cent acetone in phase 2	12·0	2·0

The film drainage was uniform and symmetrical in each case, and the minimum thickness of the film at rupture was < 500 Å. Lateral oscillations, or 'kicks'[9–11], were observed during the rise of a drop of water through cinnamaldehyde containing 1 per cent acetone. The oscillation became more marked as the drop approached the interface and continued during the early period of film thinning. Despite the fact that kicks were observed to propel the drop distances of several diameters on the interface, the circular patterns of interference colours and rings were undisturbed, indicating that film thinning continued at a uniform rate.

This work was conducted with financial assistance from the Defence Research Board of Canada (*DRB* grant 9510–05).

G. D. M. MacKay
S. G. Mason

Pulp and Paper Research Institute of Canada,
and
Department of Chemistry,
McGill University,
Montreal.

[1] Johnson, H. F., and Bliss, H., *Amer. Inst. Chem. Eng. Trans.*, **42**, 331 (1946).
[2] Allan, R. S., and Mason, S. G., *J. Colloid Sci.* (in the press).
[3] Charles, G. E., and Mason, S. G., *J. Colloid Sci.*, **15**, 236 (1960).
[4] Groothius, H., and Zuiderweg, F. S., *Chem. Eng. Sci.*, **12**, 288 (1960).
[5] Sawistowski, H. (private communication).
[6] Scriven, L. E., and Sternling, C. V., *Nature*, **187**, 186 (1960).
[7] Zuiderweg, F. S., and Harmes, A., *Chem. Eng. Sci.*, **9**, 89 (1958).
[8] Allan, R. S., Charles, G. E., and Mason, S. G., *J. Colloid Sci.*, **16**, 150 (1961).
[9] Lewis, J. B., and Pratt, H. R. C., *Nature*, **171**, 1155 (1953).
[10] Haydon, D. A., *Proc. Roy. Soc.*, **243**, 483 (1958).
[11] Davies, T. V., and Haydon, D. A., *Proc. Roy. Soc.*, A, **243**, 492 (1958).

Formation of Calcium Chloraluminate by Interaction of Portland Cement with Sea-water and Chloride Solutions

The effect of chloride-containing solutions on Portland cement has received relatively little attention. Batta[1] and Thorvaldson[2] noted that addition of chloride ions to solutions containing sulphate reduced or inhibited the expansion of concrete and mortar bars. Batta attributed this effect to the increased solubility of gypsum and calcium sulphoaluminate in chloride solutions.

In the present investigation prisms of dilute pastes of Portland cement I and V and of mortar were exposed to various attacking solutions, including Mediterranean sea-water, solutions containing 3 per cent sodium chloride + 0·4 per cent magnesium sulphate (artificial sea-water), 3 per cent sodium chloride + 0·4 per cent sodium sulphate, 0·4 per cent magnesium sulphate, 0·4 per cent sodium sulphate, 3 per cent sodium chloride and 3 per cent magnesium chloride respectively and water from the Dead Sea. The products after different reaction times were identified by X-ray powder methods and simultaneously the expansion of duplicate prisms was measured.

Contrary to observations of Batta and Thorvaldson, addition of chloride ions to solutions containing sulphate was found to increase expansion of cement and mortar bars in all the present experiments. Expansion in sodium chloride solution only slightly exceeded that in distilled water. A considerable increase of volume was observed in magnesium chloride solution but can probably be attributed to the accelerated hydration caused by the reaction of magnesium ions with calcium hydroxide and hydrated calcium silicates of the cement.

X-ray patterns of all samples exposed to chloride-containing solutions showed, besides many other lines, a strong one at 7·90–7·97 Å. It was invariably observed independent of any other components present and was attributed to $3CaO.Al_2O_3.CaCl_2.10H_2O$ (A.S.T.M. card No. 2–0081). This was confirmed by direct comparison of the X-ray patterns with that of synthetic material.

The amount of chloraluminate formed is appreciable, increasing steadily with time of exposure of the bars to chloride solutions. The variations observed in

the longest spacing, though small, are genuine and probably correspond to minor changes in composition.

The effect of the formation of chloraluminate on the expansion of cement is uncertain. The specific gravity of $3CaO.Al_2O_3.CaCl_2.10H_2O$ (1·89) is lower than that of gypsum or hydrated calcium silicates, but higher than that of $3CaO.Al_2O_3.3CaSO_4.31H_2O$. Moderately increased expansion in chloride-containing solutions might thus be anticipated, in agreement with the majority of the present experiments. However, the possible effect of chloride ions on the solubility of calcium sulphoaluminate, which was detected on all the photographs, and on its further course of reaction (to be discussed elsewhere) cannot be neglected.

The discrepancy between our results and those of Batta and Thorvaldson may perhaps be interpreted in terms of two competing factors: (*a*) the crystallization of chloraluminate; (*b*) the effect of chloride ions on the stability of other components, particularly sulphoaluminate. Their relative importance depends on the experimental conditions and remains to be investigated.

The very small expansion of cement bars in sodium chloride solution indicates that the increase of volume accompanying chloraluminate crystallization alone was largely absorbed by the pores. Only in the presence of sulphate solutions was excessive expansion observed, due to the combined effect of the formation of calcium chloraluminate and calcium sulphoaluminate.

Failure of previous investigators to detect chloraluminate in the course of their many studies of attack of cement by sea water may perhaps be attributed to the lack of X-ray data in relevant cases.

We wish to express our thanks to the Ford Foundation for a grant which made this research possible.

L. Heller
M. Ben-Yair

Standards Institution of Israel,
Tel Aviv,
and Geological Survey of Israel,
Jerusalem.

[1] Batta, G., *Ann. Trav. Pub. Belg.* (August 1948).
[2] Thorvaldson, T., *Proc. Third Intern. Symp. Chem. Cement* (London, 1952).

Chromium, Uranium, Tungsten and Nickel Complexes of Thioglycolic Acid

The most commonly available of the thiol-acids is thioglycolic acid, also known as mercapto-acetic acid. This reagent is favoured by analytical chemists in research and industry[1-3] for the colorimetric estimation of iron in micro-quantities because of certain advantages it offers[4,5]. In this Laboratory this reagent had been used in the annulus method for separation and detection of chromium and titanium on a filter paper[6].

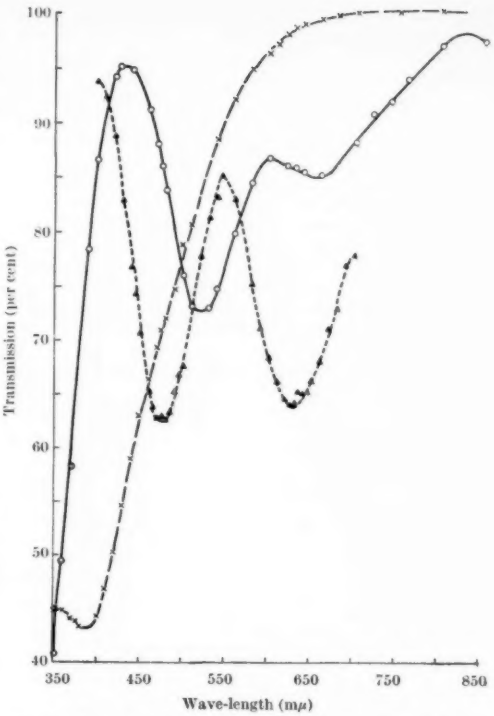


Fig. 1. Transmission curves of the thioglycolic acid complexes. ·, chromium, 30 μgm. Cr/ml. at *p*H 9, light-path 40 mm.; ×, uranium, 10 μgm. U/ml. at *p*H 7, light-path 40 mm.; ○, nickel, 23·5 μgm. Ni/ml. at *p*H 7, light-path 40 mm.

Chromium has been usually determined colorimetrically, for example, with diphenyl carbazide, after oxidizing it to the hexavalent state, but the use of thioglycolic acid has the distinct advantage that chromium can be present in the original solution in the hexa- or tri-valent state. The use of this complexing agent has been extended for the colorimetric determination of the other elements of Group VI *A*, namely, tungsten and uranium, and also to nickel which frequently occurs in admixture with these. This reagent has already been used for the estimation of molybdenum[7] alone or in the presence of

Table 1

	Chromium	Uranium	Tungsten	Nickel
Colour of complex in acidic medium	Violet	Colourless	Yellowish	Yellowish-green
Absorption maxima	350 mμ (*p*H 2)	—	(10^{-3} *M* soln.)	(10^{-2} *M* soln.) (*p*H 2)
Colour of complex in alkaline medium	Green	Orange	Colourless	Pink
Working range of *p*H	(6–9)	(6·5–7·5)	(2·5–5·5)	(7–9)
Absorption maxima	*630 mμ and 475 mμ	388 mμ	305 mμ	522 mμ
Molar absorbency index (A_M)	$1·95 \times 10^2$ (on either max.)	23×10^2	53×10^2	$0·98 \times 10^2$
Approximate ratio of A_M	2	25	55	1
Limits of determination (absorptiometrically)	$(1–75) \times 10^{-4}$ *M*	$(2–75) \times 10^{-5}$ *M*	$(4–40) \times 10^{-6}$ *M*	$(2–20) \times 10^{-4}$ *M*
Polarographic $E_{1/2}$ versus S.C.E.	—	—	—	−0·68 V.
Limits of determination (polarographically)	—	—	—	$(0·2–2·0) \times 10^{-4}$ *M*

* In industrial analysis one could use a Spekker absorptiometer and a combined filter (ref. 12) consisting of one filter from Chance's glass-filter set *H*455 and one from Ilford gelatin-filter, namely (OB_2 + 602) and (OY_2 + 607) respectively for 475 mμ and 630 mμ over the same range of concentration with a 40-mm. absorption cell.

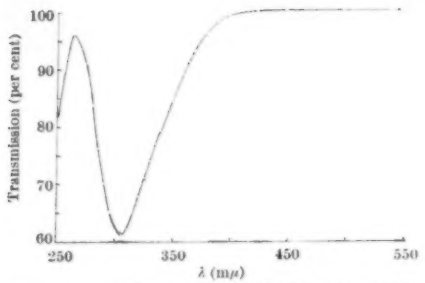


Fig. 2. Transmission curve of thioglycolic acid complex of tungsten. ~ 15 μgm. W/ml. at *p*H 3·5, light-path 10 mm.

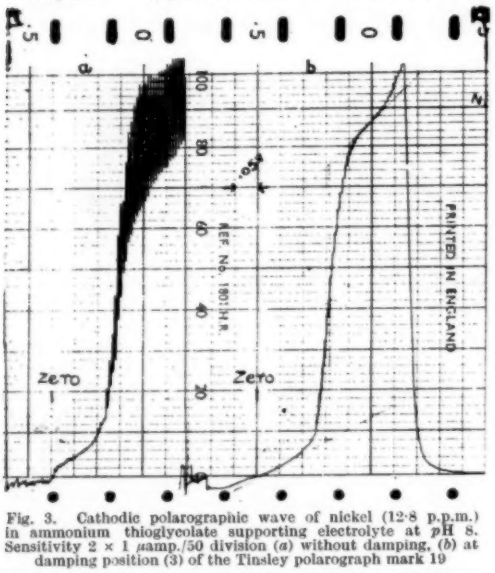


Fig. 3. Cathodic polarographic wave of nickel (12·8 p.p.m.) in ammonium thioglycolate supporting electrolyte at *p*H 8. Sensitivity 2 × 1 μamp./50 division (*a*) without damping, (*b*) at damping position (3) of the Tinsley polarograph mark 19

tungsten[8]. Thioglycolic acid was used[9] as a complexing agent for uranium, but the effect of variation of *p*H has been critically investigated only recently and an absorption maximum reported[10]. The nickel complex has also been investigated polarographically[11] and since the reduction wave is favourably placed at −0·18 V. against a mercury pool, interference from other elements in the polarographic determination of nickel is excluded. The reaction at the dropping mercury electrode involves a two-electron reduction. The wave-height is diffusion-controlled and the temperature coefficient of the wave-height is about 2 per cent per degree rise of temperature. The effect of variation of *p*H and the interference of other ions has been determined in the colorimetric and polarographic determinations of chromium, tungsten, uranium and nickel. Of these, the complexes of thioglycolic acid with chromium, tungsten and nickel have been investigated for the first time.

In a typical determination the solution is treated with excess of reagent on a hot plate and the *p*H of the solution is then adjusted to the required value. After re-heating, the *p*H is checked again. The solution of the complex is then analysed for the respective element. The results of some typical determinations are shown in Table 1. The transmission spectra of different complexes and the reduction wave of the nickel complex[11] with thioglycolic acid are shown in Figs. 1–3.

The complexes of chromium, tungsten, uranium and nickel can be used for the micro-determination of these metals. They are quite stable (the tungsten complex is stable for two weeks), and even a large excess of the thioglycolic acid can be used without any detrimental effect, which is not the case with thiocyanate complexes of tungsten, uranium, etc. Nickel also complexes with *o*-mercaptobenzoic acid, a homologue of thioglycolic acid, which has similar absorption characteristics to nickel thioglycolate. The reagent has been found to be a promising complexing agent for other metals, for example, rhenium, indium, etc. Complete results of these investigations will be communicated later.

I wish to acknowledge the collaboration of Dr. J. A. W. Dalziel, of the Imperial College of Science and Technology, London, in the polarographic investigation of nickel.

V. M. Bhuchar

National Physical Laboratory,
New Delhi-12, India.

1 Welcher, F. J., *Organic Analytical Reagents*, **4**, 159 (D. Van Nostrand, New York, 1948).
2 Flegl, F., and Caldas, A., *Anal. Chem.*, **23**, 8 (1951).
3 *The Analysis of Titanium and its Alloys*, 47 (Imperial Chemical Industries, Ltd., London, 1959).
4 Swank, J. W., and Mellon, M. G., *Indust. Eng. Chem.*, Anal. Ed., **10**, 7 (1938).
5 Sandell, E. B., *Colorimetric Determination of Traces of Metals*, second ed., 364 (Interscience, 1950).
6 Bhuchar, V. M., and Verma, M. R., *Mikrochim. Acta*, 342 (1958).
7 Will, Fritz, III, and Yoe, J. H., *Anal. Chem.*, **25**, 1363 (1953).
8 Otterson, D. A., and Graab, J. W., *Anal. Chem.*, **30**, 1282 (1958).
9 Davenport, W. H., and Thomason, P. F., *Anal. Chem.*, **21**, 1093 (1949).
10 Bhuchar, V. M., see *Adv. in Polarography*, **2**, 537; *Proc. Second Intern. Cong. Polarography*, Cambridge (1959), edit. by I. S. Longmuir (Pergamon Press, Oxford, 1960).
11 Bhuchar, V. M., and Dalziel, J. A. W. (presented to the Second Seminar on Electrochemistry, Central Electrochemical Research Institute, Karaikudi, India, 1960).
12 Haywood, F. W., and Wood, A. A. R., *Metallurgical Analysis with Spekker Absorptiometer*, 20 (Hilger and Watts, London, 1957).

BIOCHEMISTRY

Interaction of Hydrocortisone, the Sulphydryl Group, and the Catechol Amines

Hydrocortisone potentiates the physiological effects of epinephrine and norepinephrine[1]. The mechanism of this action remains obscure. Recently, reaction of oxidation products of the catechol amines with the sulphydryl group has been demonstrated *in vitro*[2,3]. Further *in vitro* studies suggest that the physiological interrelationship between hydrocortisone and the catechol amines may involve the sulphydryl group.

The norepinephrine, cysteine and manganese dioxide used were previously described[2,4]. Steroids were obtained from the Upjohn Co., the Schering Corporation and the Nutritional Biochemical Corporation. After solution of approximately 10 mgm. of hydrocortisone in 1·0 ml. of absolute ethyl alcohol, the concentration was decreased to 500γ per ml. by addition of water. The other steroids studied were used in solution in absolute alcohol only. Other reagents included 2/3 *N* sulphuric acid and 1 *N* sodium hydroxide. Buffers were 0·1 *M* sodium phosphate of *p*H 7·4 and 8·0. Absorbencies were measured in the Beckman *DU* spectrophotometer with tungsten lamp.

The experimental procedure began with the preparation of a solution containing 0·15 × 10^{-3} *M* cysteine

and the indicated concentration of hydrocortisone immediately before its utilization. At zero time, 0·5 ml. of 1 *N* sodium hydroxide was added to separate test-tubes containing 2·0-ml. aliquots of this solution. One-half of these tubes were used for the development of noradrenochrome, the other half for the simultaneous measurement of the residual sulphydryl group. At 5-min. intervals after the alkalinization, 0·70 ml. of 2/3 *N* sulphuric acid, 1·0 ml. of 0·1 *M* sodium phosphate buffer of *p*H 7·4, 0·1 ml. of 3·0 × 10^{-3} *M* norepinephrine, and 0·1 gm. of manganese dioxide were added rapidly to an additional tube and oxygen was bubbled vigorously through the solution for 40 sec. 6 min. later, the absorbency of the noradrenochrome formed was measured at 480 mμ. Simultaneously, every 5 min., 0·68 ml. of 2/3 *N* sulphuric acid and 1·0 ml. of 0·1 *M* sodium phosphate buffer of *p*H 8·0 were added to a separate tube. The sulphydryl group was then determined from the absorbency at 412 mμ of the compound formed by Ellman's technique[5]. Parallel tubes with cysteine but without hydrocortisone were used as controls in each case.

Table 1. PERCENTAGE OF THE SULPHYDRYL GROUP OF CYSTEINE REMAINING IN THE FREE STATE AFTER VARIOUS PERIODS OF TIME

Molarity of hydrocortisone / Molarity of cysteine	6 min.	15 min.	30 min.
0 (auto-oxidation))	91	84	74
½	84	69	47
1	77	58	26
3	60	25	0

Reagent solutions containing 500γ of hydrocortisone per ml. of alcohol alone or per ml. of alcohol and water as described affected the sulphydryl group identically, the effect of the alcohol being negligible. The effects of various concentrations of hydrocortisone upon the sulphydryl group of cysteine in alkaline solution are presented in Table 1. In contrast to hydrocortisone such steroids as progesterone, testosterone and deoxycorticosterone acetate had no effect, while corticosterone was only slightly effective. On the other hand, 17α-hydroxy-11-deoxycorticosterone, cortisone and hydrocortisone acetate had essentially the same effect as hydrocortisone. This action of the steroids was present only in highly alkaline solution. The results for glutathione were similar in direction but considerably less in magnitude than those for cysteine. The amount of noradrenochrome developed from norepinephrine at *p*H 7·4 increased as the free —SH group decreased. The intensity of the pink colour and the absorbency at 480 mμ reflected the disappearance of the sulphydryl group, just as did the Ellman technique.

The experimental evidence suggests that the sulphydryl group of cysteine reacts in alkaline solution on an equimolar basis with many steroids which have a C_{17} side-chain. The reactivity of hydrocortisone acetate indicates that the C_{21} hydroxyl group is not essential. Conversely, the ineffectiveness of progesterone and deoxycorticosterone acetate shows the inadequacy of the C_{20} oxygen alone. The presence of the keto-group on C_{20} and the α-hydroxyl group on the C_{17} position is characteristic of the steroids which react with the sulphydryl group. It is interesting to note that this high reactivity of cortisone and hydrocortisone *in vitro* parallels their strong potentiation of catechol amir ' action *in vivo*[1].

The increased development of noradrenochrome at *p*H 7·4 as the cysteine disappears is important because it illustrates a possible mechanism of hydrocortisone action *in vivo*. The norepinephrine–cysteine and norepinephrine–glutathione compounds have no effect upon the blood pressure of the dog, according to recent work done at the University of Louisville in conjunction with Dr. K. C. Huang of the Department of Pharmacology. Hence, by preventing combination of cysteine and glutathione with derivatives of the catechol amines, hydrocortisone might direct the metabolic pathways of epinephrine and norepinephrine toward reactions which would intensify and prolong their physiological effects. An enzyme which would facilitate the reaction of the sulphydryl group and hydrocortisone at *p*H 7·4 is not available as yet. The existence of such an enzyme appears to be quite feasible, and search for it *in vivo* is under way.

This investigation was supported by grant No. *M*-2795 (*C*1) from the National Institutes of Health of the U.S. Public Health Service.

SIDNEY ROSTON

Department of Medicine,
University of Louisville,
School of Medicine, Kentucky.

[1] Ramey, E. R., and Goldstein, M. S., *Physiol. Rev.*, **37**, 155 (1957).
[2] Roston, S., *Arch. Biochem. Biophys.*, **85**, 74 (1959).
[3] Bouchilloux, S., and Kodja, A., *C.R. Acad. Sci.*, **251**, 1920 (1960).
[4] Roston, S., *J. Biol. Chem.*, **235**, 3315 (1960).
[5] Ellman, G. L., *Arch. Biochem. Biophys.*, **74**, 443 (1958).

Effects of Injections of Some Intermediary Metabolites on the 'Insulin Response' of Normal Rabbits

CONTINUED daily injections of β-hydroxy butyrate in gradually increasing doses has been found to increase initially the insulin content of the pancreases of guinea pigs and then bring about a gradual decrease of active insulin/gm. of pancreas tissue[1]. Similar experiments by Schwartz *et al.*[2] have recently confirmed these findings in both guinea pigs and albino-rats. The present communication indicates the effects of some members of the trichloroacetic acid cycle, compared to β-hydroxy butyrate, on the insulin response of normal rabbits.

Pure sodium salts of the metabolites concerned and crystalline insulin solution (Lily) were used in these experiments on healthy normal rabbits. Five rabbits were used in each case and each experiment was repeated twice as in cross-over tests in insulin assay[3]. Each rabbit was allowed one week's rest between experiments.

In one set of experiments sodium salts of the metabolites concerned (dissolved in 5 c.c. of normal saline) were injected in fasting (18 hr.) normal rabbits and the blood sugar-level studied at hourly intervals for a period of 3 hr. In a second set of experiments the metabolites concerned were mixed *in vitro* with 2 units of insulin (diluted to 5 c.c. with normal saline), the mixture incubated at 37° C. for 30 min., and the clear solution injected in one leg of the rabbits. The blood sugar-level was then examined as in the previous case. In a third set of experiments the metabolites were injected simultaneously but separately with 2 units of insulin in the two legs of the rabbits to study the *in vivo* effect on the insulin response. Control experiments were carried out simultaneously with each set of the above experiments to study the blood sugar response on injection of 2 units of insulin alone. The micro-method of Folin and Malmros[4] was used in estimating blood sugar in samples of blood drawn from the marginal ear veins of the experimental rabbits. The results of these experiments are given in Table 1.

XUM

Table 1. Effects of Injections of Metabolites on the Insulin Response of Normal Rabbits

S. No.	Substance injected	Average percentage of reductions in blood sugar			
		2 units insulin only	100 mgm. of substance	100 mgm. and 2 units insulin *in vivo*	100 mgm. and 2 units insulin *in vitro*
1	Insulin (control)	56·9 ± 3·46	—	—	—
2	Sodium pyruvate	—	8·98 ± 1·1	66·78 ± 3·2	50·8 ± 3·4
3	Sodium β-hydroxybutyrate	—	12·84 ± 1·5	66·75 ± 3·5	53·43 ± 2·1
4	Sodium isocitrate	—	17·12 ± 2·0	57·51 ± 3·3	49·0 ± 2·8
5	Sodium succinate	—	22·54 ± 1·8	56·14 ± 3·2	46·0 ± 3·0
6	Sodium citrate	—	30·34 ± 3·0	51·98 ± 3·1	40·83 ± 2·8

From Table 1 it is apparent that all these metabolites when injected alone in normal fasting rabbits produce a hypoglycæmic response which may be due to their stimulating effect, direct or indirect, on the *B*-cells of the islets of Langerhans, initiating a secretion of insulin. This stimulation for the same amount of substance injected is greatest in case of citrate and is progressively lesser in the following order: citrate–succinate–isocitrate–β-hydroxy butyrate–pyruvate.

The results of *in vivo* experiments suggest that while the stimulating power of β-hydroxy butyrate and pyruvate are independent of the presence of exogenous insulin in the system, the other three metabolites, namely, citrate, succinate and isocitrate, seem to lose their stimulating power in presence of exogenous insulin in the system. The results of *in vitro* experiments suggest that while β-hydroxy butyrate and pyruvate have no effect on the biological potency of exogenous insulin, the other three metabolites, namely, citrate, succinate and isocitrate, bring about a decrease in the biological potency of exogenous insulin in similar circumstances, namely, *in vitro* mixing and incubation at 37° C. for 30 min. before injection.

H. D. Brahmachari
G. Raghupathy Sarma

Biochemistry Laboratory,
Birla College,
Pilani (Rajasthan).

[1] Brahmachari, H. D., and Mahendra Kumar, *Nature*, **183**, 51 (1959).
[2] Schwartz *et al.*, *Nature*, **185**, 771 (1960).
[3] Marks, *League of Nations Rep. Insulin Standardization*, C.H. 396 (1928).
[4] Folin and Malmros, *J. Biol. Chem.*, **83**, 115 (1929).

Estimation of Protein in Cellular Material

Protein content is a widely used reference value for microbial growth yield, enzyme activity, and other biochemical quantities. The assay of dissolved protein is relatively easy, being accomplished by measuring the absorbence at 280 mμ or by use of the biuret reagent, etc. The assay of protein in cellular material involves a time-consuming digestion (Kjeldahl method). In addition to being slow, this method is non-specific and does not immediately distinguish between proteins and other nitrogen compounds.

The method reported here is based on the fact that most solid protein samples, including precipitates made with trichloroacetic acid or by heat coagulation, are soluble in a dilute base. By using half-strength biuret reagent (containing 0·4 *M* sodium hydroxide) it is possible to extract and afterwards measure rapidly.

Cell materials seem to fit three categories with regard to method: (1) Require no previous treatment (most bacteria). (2) Chlorophyllous cells (algae and leaf tissue) require extraction with 70–80 per cent ethanol to remove pigments. (3) Cells that resist base extraction of their protein (yeast and algae). These require pre-treatment with 0·5 *M* perchloric acid. The reason for this is not clear but may be related to cell wall thickness or to covalent bonds existing between the protein and structural polymers. Nevertheless, pre-treatment with perchloric acid does make reproducible protein extraction possible. (The bean leaf tissue does not require pre-treatment with perchloric acid but was so treated for other reasons.)

Wet cell masses are collected by centrifugation and washed, if necessary, to remove any large amount of reducing sugar which may be present in the medium. (The cupric ions in the biuret reagent are susceptible to reduction and precipitation as cuprous oxide.) The centrifuged solids are suspended in at least 10 vol. of water to which is added an equal volume of biuret reagent[1]. This mixture is allowed to stand at room temperature for one hour with occasional stirring. The insoluble residue is removed by centrifugation, leaving a water-clear, purple solution. Readings are made in tubes (16 mm. int. diam.) against a reagent blank in a Coleman spectrophotometer at 540 mμ.

Proteins from different sources give somewhat different colour yields. Thus, an absolute protein measurement must be made if it is necessary to calibrate the method. (Such calibration is not necessary when comparisons are to be made only between samples of the same biological origin.) In the examples presented here the protein was first read as a biuret colour intensity and then precipitated with trichloroacetic acid. The precipitate is analysed via Kjeldahl technique and the usual factor of 6·25 mgm. protein/mgm. N is applied. It is assumed that trichloroacetic acid brings down all the protein. This is apparently borne out by analyses which show that only a small amount of nitrogen (attributable to compounds of low molecular weight) remains in the frac-

Table 1. Colour Yields and Extraction Efficiency of Cellular Protein with Biuret Reagent

Cell material	Colour yield O.D. × ml./mgm. protein	Percentage of TCA-insoluble nitrogen in extractable nitrogen	Percentage of non-extractable nitrogen (based on extractable plus non-extractable nitrogen)
Bean leaf* *Phaseolus vulgaris*	0·59	96	3
Algae* *Chlorella vulgaris*	0·55	83	29
Yeast† *Saccharomyces cerevisiae*	0·54	91	24
Bacteria *Azotobacter vinelandii*	0·48	84	10
Bacteria *Escherichia coli*	0·39	85	22

* Pre-treated with ethanol and then perchloric acid.
† Pre-treated with perchloric acid.

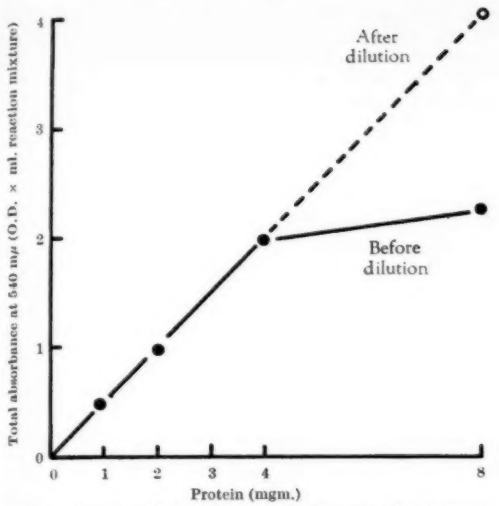


Fig. 1. Linearity of biuret colour intensity versus protein concentration (*A. vinelandii*)

tion soluble in trichloroacetic acid. Table 1 illustrates some representative results.

The biuret reagent does not have a wide range of linearity. Furthermore, the solubilized protein from different sources appears to have individual ranges of linearity. For both these reasons it is well to establish first a colour-concentration curve for each type of material. Fortunately, it is possible to dilute non-linear samples with a 1 : 1 mixture of water and reagent. After an additional incubation of 30 min. the colour is found to be proportional to protein (Fig. 1).

Extraction of protein may not be quantitative for all materials (Table 1). This may not be a serious problem to persons concerned with only one type of cell, especially when repeated extractions yield results within ±5 per cent. In the case of yeast cells the unextracted nitrogen may be a 'fungal chitin'. In the case of *E. coli* at least some of the unextracted nitrogen is protein, as judged by the biuret-purple colour of the remaining solids. This particular extraction might be made more quantitative by pretreatment with 0·5 *M* perchloric acid.

Chlorophyll-containing materials are extracted in boiling 70 or 80 per cent ethanol until colourless. The solids are then extracted with perchloric acid and/or biuret reagent.

The centrifuged material is suspended in at least 10 vol. 0·5 *M* perchloric acid and incubated, with occasional stirring, at 80° for 90 min. (This is essentially the method used by Basler and Commoner for extraction of ribonucleic acid[3].)

The combined loss of protein due to the treatments with dilute biuret reagent and 0·5 *M* perchloric acid is less than 10 per cent (ref. 3).

David Racusen
D. B. Johnstone

Department of
Agricultural Biochemistry,
University of Vermont,
Burlington.

[1] Cornell, A., Bardawill, C., and David, M., *J. Biol. Chem.*, **177**, 751 (1949).
[2] Basler, E., and Commoner, B., *Virology*, **2**, 15 (1956).
[3] Racusen, D., and Foote, M., *Arch. Biochem. Biophys.*, **90**, 90 (1960).

1-(Indole-3-acetyl)-β-D-Glucose, a New Compound in the Metabolism of Indole-3-acetic Acid in Plants

Studying the fate of exogenously applied indole-3-acetic acid (IAA) in pea epicotyls, we observed, besides indoleacetylaspartic acid[1] (IAAsp), a trace amount of a compound which showed definitely indole reactive properties and which was split on alkaline hydrolysis into IAA. The amount of this metabolite proved too small for identification, therefore we carried out a rough survey on one species from 20 plant families using 2-^{14}C IAA in the hope of finding an accumulation of this metabolite. This unknown compound was found in the leaves of the monocotyledonous plant *Colchicum neapolitanum* Ten. to about 65 per cent of the total amount of IAA taken up, the rest being unchanged IAA (20 per cent) and minor components (Fig. 1, top). IAAsp was detected, if at all, only in very small quantities. In order to isolate a larger amount of this compound, 70 gm. of mature *Colchicum* leaves were cut into pieces and incubated with 1 litre of a 4 × 10^{-4} *M* solution of IAA for 24 hr. with good aeration. The compound was extracted from the leaves with boiling 80 per cent aqueous ethanol; the extract was then concentrated to a small volume and made up to a powder with 'Hyflo supercel' and then extracted in a Soxhlet-type extractor for 6 hr. with ether. The ether containing only the IAA was discarded, and extraction continued with ethyl acetate for another 6 hr. This solution was taken to dryness and the residue containing the compound chromatographed as streaks on Whatman No. 3 paper in *n*-butanol / glacial acetic acid / water (5 : 1 : 2·2, R_F 0·56) and in *iso*propanol / benzine / water (55 : 30 : 11, R_F 0·39). By elution and rechromatography between the two solvents, the derivative could be purified and resulted finally in 6·05 mgm. of a highly purified, non-crystalline material. The compound was located on the paper by spraying with *p*-dimethylaminobenzaldehyde.

Hydrolysis of the material with 1 *N* potassium hydroxide at room temperature led to the hydrolysis products IAA and a carbohydrate chromatographically identical with glucose. Acid hydrolysis with 2 *N* hydrochloric acid at 100° C. gave the same result. The compound was split with hydroxylamine to form indoleacetyl hydroxamic acid, chromatographically identical with synthetic hydroxamate, thus showing an ester linkage.

The ultra-violet absorption spectrum of the compound showed a minimum at 248 mμ, a maximum at 278 mμ and a shoulder at 288 mμ. Equimolar solutions of IAA and the unknown had the same extinction coefficient at 280 mμ while the differences in the spectra of the two compounds showed a maximum at 240 mμ (E = 2·9 × 10^3). This spectrum closely resembles that of indoleacetyl-thioesters[2], thus again suggesting an ester linkage between IAA and glucose. On treatment with the Salkowski reagent[3], the resulting colour of the compound was shifted from the IAA peak ($\lambda_{max.}$ 535) towards the longer wave-length ($\lambda_{max.}$ 545); indolethylacetate shows $\lambda_{max.}$ 555 and indoleacetyl-thioesters, $\lambda_{max.}$ 575. Maximal colour intensity was attained in each case 11 min. after the addition of the reagent. Aliquots of the compound were analysed for functional groups and the ratio of the assays was IAA / ester linkage / glucose = 1·00 : 1·01 : 1·00. The IAA concentration was based on the absorption at 280 mμ, glucose was determined with the anthrone reagent[4] and the ester linkage with

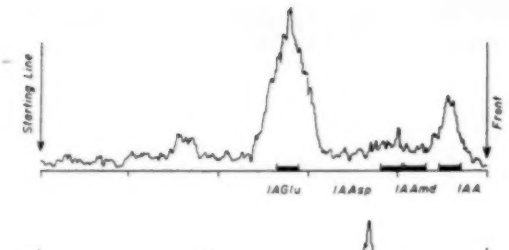


Fig. 1. (Top): Scanning record of a chromatogram of an alcoholic leaf-extract after 24 hr. treatment with 10^{-4} *M* 2-^{14}C IAA developed in *n*-butanol / acetic acid / water (5 : 1 : 2·2). (Bottom): Scanning record of a chromatogram with the same extract developed in *iso*propanol / NH_3 (25 per cent) / water (8 : 1 : 1)

the hydroxamic acid test[5]. In the absence of free IAA, 1 μ*M* of indoleacetyl hydroxamate in 5 ml. test solution gives an extinction of 0·230 Beckman unit at 540 mμ. On the basis of this analysis the compound represents a glucose ester of IAA. To determine which carbon atom of glucose is esterified, the compound was subjected to the action of β-glucosidase (K^+, acetate buffer, *p*H 5·0) and was split quantitatively by this enzyme. The compound lacks reducing properties. These properties are characteristic for an ester linkage in the 1-position of glucose in β-configuration. I am therefore able to assign to the ester the structure of 1-(indole-3-acetyl)-β-D-glucose (IAGlu). (During the preparation of this manuscript a tentative identification of this ester was made by Klämbt, H. A. (*Planta*, **56**, 618 ; 1961). A critical discussion of this presentation will be left to a further publication.)

The ester is labile towards ammonia. Using ammoniacal *iso*propanol for chromatography resulted in the ammonolysis of 57 per cent and in the hydrolysis of 13 per cent of the ester, a total of 70 per cent of the ester being destroyed. The predominant reaction product was indoleacetamide (IAAmd) (Fig. 1, bottom). IAGlu is also split in bicarbonate solution containing ammonia salts to yield IAA and IAAmd. I therefore assume that the reports in literature on the occurrence of IAAmd in plants are erroneous and probably due to chromatographic and isolation artefacts. This behaviour of IAGlu is in accordance with the ammonolysis of indoleacetyl-glucosiduronic acid[6].

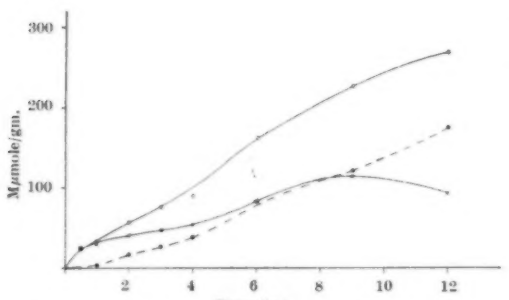


Fig. 2. The uptake of IAA from solution and accumulation of IAA and IAGlu in leaf-disks of *C. neapolitanum* during 12 hr. of incubation in a 5×10^{-5} *M* 2-^{14}C IAA solution. ○, Total uptake of IAA from external solution; ●, IAGlu, and ×, IAA, accumulating in leaf-disks

The uptake and metabolism of IAA by *Colchicum* leaf-disks is shown in Fig. 2. The uptake of IAA during 12 hr. was fairly constant. The quantity of IAA in the free form rises during the first 9 hr. and then falls off again. In contrast to experiments with pea epicotyls[7], the IAA concentration in this leaf tissue exceeded the concentration in solution by a factor of more than 2, a fact which suggests a metabolic uptake of the acid. Unlike the conjugation of IAA with aspartic acid there was no lag-period in the conjugation of IAA with glucose. The synthesis of IAGlu certainly involves a transference of glucose from uridinediphosphoglucose on to IAA.

Besides the conjugation with aspartic acid, I therefore assume a second de-toxification mechanism of aromatic acids in plants, the conjugation with glucose. Both types of de-toxification may operate simultaneously or one or the other may predominate in different plant species.

IAGlu is a member of the recently discovered group of naturally occurring sugar esters[8], and is active in the *Avena* cylinder-test.

This work was supported by the Bundesministerium für Atomkernenergie und Wasserwirtschaft.

2-^{14}C IAA was a gift from Dr. H. Simon, for which we are very grateful.

M. H. Zenk

Botanical Institute,
University of Munich,
Munich 19.

[1] Andreae, W. A., and Good, N. E., *Plant Phys.*, **30**, 380 (1955).
[2] Zenk, M. H., *Naturwiss.* (in the press).
[3] Tang, Y. W., and Bonner, J., *Arch. Biochem. Biophys.*, **13**, 11 (1947).
[4] Aronoff, S., *Techniques of Radiobiochemistry* (Iowa State College Press, 1957).
[5] Lipmann, F., and Tuttle, L. C., *J. Biol. Chem.*, **159**, 21 (1945).
[6] Jepson, J. B., *Biochem. J.*, **69**, 22*P* (1958).
[7] Andreae, W. A., and van Ysselstein, M. W., *Plant Phys.*, **35**, 225 (1960).
[8] Birkofer, L., Kaiser, C., and Kosmol, H., *Naturwiss.*, **47** 469 (1960).

Effect of Amino-Acid Esters on the Growth of Fibroblast Cultures

Perri *et al.*[1] demonstrated that amino-acid esters inhibit the growth of bacteria. Simultaneously with this finding, Horváth *et al.* described the inhibition of certain amino-acid esters on the adaptive amylase production of *Penicillium chrysogenum*[2]. Fehér *et al.* demonstrated that DL-methionine-ethyl ester caused liver lesions[3].

The mechanism of this inhibition has not yet been examined, but it is supposed that because of the bound carboxyl group the amino group becomes more reactive and thus the activated amino-acid reacts with the ester instead of the intact amino-acid.

The inhibition of ethionine on growth and protein synthesis is well known. We supposed that this effect is unable to diminish even after the esterification of ethionine. To prove this supposition, we attempted to compare the inhibition of growth by ethionine and ethionine ethyl ester on cell cultures.

The pure fibroblast culture used in our experiments was cultivated in tubes of 3 ml. volume described elsewhere[4]. An ocular micrometer was used to measure

the radii of the cell cultures. The basis of comparison was the total of radii measured in four directions. The basic growth of the cultures was measured always on the third day after the inoculation of the cells and the substance investigated was added in a volume of 0·1 ml. to the culture medium. The difference between the basic growth and that on the third day of the experimental period characterized the activity of the substances examined.

In the first experiment we investigated whether certain amino-acid esters inhibit the growth of tissue cultures similarly to bacteria described previously[4,5]. 1·5 m.mole/l. methionine ethyl ester was added to the culture medium. An inhibition of more than 50 per cent was observed (Table 1). The inhibition of methionine anti-metabolites, as is well known, can be prevented by methionine or glutamine[3,6]. Next we added 3 m.mole/l. methionine or glutamine to the culture medium, which prevented the inhibition of methionine ethyl ester about 50 per cent. Methionine ethyl ester behaves thus like other methionine antagonists and its growth inhibition may be prevented by addition of methionine to fibroblast cultures.

Table 1. Effect of Amino-Acid Esters on the Growth of Fibroblast Cultures

Added substance	No. of cultures	Basic growth	Growth after addition of substance	Growth in percentage compared with the control
Control	520	24·0 ± 5·5	45·0 ± 9·2	100
DL-Methionine-ethyl ester, 0·15 m*M*	440	24·3 ± 5·3	34·0 ± 7·1	45·7
DL-Methionine-ethyl ester, 0·15 m*M* + DL-Methionine, 0·3	385	22·1 ± 4·0	37·4 ± 6·2	72·5
Control	114	29·4 ± 4·4	52·2 ± 5·3	100
DL-Methionine-ethyl ester, 0·15 m*M*	120	29·8 ± 3·8	37·2 ± 5·0	32·0
DL-Methionine-ethyl ester, 0·15 m*M* + DL-Glutamine, 0·3 m*M*	126	28·7 ± 5·7	43·4 ± 6·9	64·5
Control	343	20·4 ± 3·6	36·0 ± 6·9	100
DL-Ethionine-ethyl ester, 1·5 m*M*	163	19·5 ± 4·1	33·7 ± 6·2	89·0
DL-Ethionine, 1·5 m*M*	202	19·6 ± 3·9	25·9 ± 5·4	39·4
DL-Ethionine, 1·5 m*M* + DL-Ethionine-ethyl ester, 1·5 m*M*	209	18·9 ± 3·7	31·8 ± 6·7	80·5

Further, we studied the ethionine sensitivity of the culture. As shown in Table 1, 1·5 m.mole/l. ethionine causes a significant inhibition of growth.

On the basis of the hypothesis discussed here, it might have been expected that ethionine ethyl ester added in the same concentration of ethionine produces inhibition of growth similar to ethionine or methionine ethyl ester. In the next experimental series we therefore added ethionine ethyl ester in a concentration of 1·5 m.mole/l. to the culture medium. The ester was practically ineffective, the inhibition being only 11 per cent.

Due to simultaneous addition of a double amount of ethionine ethyl ester (3 m.mole/l.) and of 1·5 m.mole/l. of ethionine, the inhibition of growth by ethionine decreased about 50 per cent.

It seems that the inhibition of methionine ethyl ester was of a competitive character, because it could be prevented by addition of methionine in greater amounts. In contrast with expectation, however, the inhibition of the ethionine and ethionine ethyl ester added at the same time is not additive, but the ester nearly prevents the inhibition. The quantitative conditions point to competitive inhibition, too, although in this case it is the ester that prevents the effect of the amino-acid.

Our experiments do not explain the mechanism of inhibition of amino-acid ester, but it seems that the hypothesis that inhibition was induced only by the increased reactivity of the amino-group is dubious.

I. Fehér*
A. Döklen
V. Selmeci

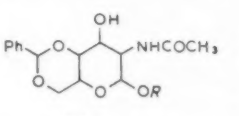
Institute of Pathophysiology,
University Medical School,
Budapest.

* Present address: Inst. for Radiobiol. Res., Budafok.

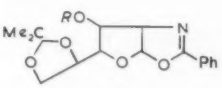
[1] Perri, C. C., Kaplan, L., and Stock, C. C., *Nature*, **183**, 116 (1959).
[2] Horváth, I., Bajusz, S., and Szentirmai, A., *Nature*, **183**, 477 (1959).
[3] Fehér, I., Selmeci, F. V., and Kemény, T., *Nature*, **187**, 422 (1960).
[4] Döklen, A., Fehér, I., and Sós, J., *Acta Physiol. Hung.* (in the press).
[5] Fehér, I., Lengyel, A., and Nász, I., *Acta Physiol. Hung.*, **15**, 275 (1958).
[6] Rabinovitz, M., Olson, M. E., and Greenberg, D. N., *J. Biol. Chem.*, **222**, 217 (1957).

A Convenient Synthesis of Muramic Acid and Other 3-O-Ethers of D-Glucosamine

3-O-Ethers of D-glucosamine, including muramic acid (3-O-carboxyethyl-D-glucosamine, a component of bacterial cells walls), have been prepared[1–4] from the intermediate I (R = Me or Et)[5]. We have applied the Williamson ether synthesis to the intermediate II (R = H)[6] and have prepared muramic acid, 3-O-methyl- and 3-O-carboxymethyl-D-glucosamines by this procedure. The intermediate (II) is readily prepared in good yield by a two-stage process, whereas the preparation of substance I requires three steps and a separation of the α and β anomers[7,8].



(I)



(II)

Compound II (R = H) was prepared by the method of Konstas, Photaki and Zervas[6] except that the crude N-benzoyl-D-glucosamine which separates from the reaction mixture was used (after thorough drying over phosphorus pentoxide *in vacuo*) without further purification. The overall yield of crystalline II (R = H, m.p. 158–160°) from glucosamine hydrochloride was 55 per cent. After recrystallization from ethyl acetate, the compound had the following characteristics: m.p. 160–162°; $[\alpha]_D^{19}$ + 6° (*c*, 2 in chloroform); infra-red absorption (potassium chloride disk) 1,648 cm.$^{-1}$ (C=N stretching vibration).

For the synthesis of muramic acid, intermediate II (R = H) (35 gm.) was treated with sodium hydride (2·9 gm.) in refluxing tetrahydrofuran (450 ml., sodium-dried, peroxide-free) for 2 hr. To the cooled solution of the sodio derivative was added dried, redistilled, ethyl DL-α-bromopropionate (35 gm.). There was an immediate precipitation of sodium bromide, and after the initial exothermic reaction had subsided, refluxing was continued for another hour. After cooling, the solution was filtered and the tetrahydrofuran and excess bromo-ester were removed by

evaporation under reduced pressure. The crude product was saponified by warming for 5 min. at 50° in a mixture of water (35 ml.), methanol (35 ml.) and sodium hydroxide (8 gm.). This solution was then diluted with water (350 ml.), saturated with potassium chloride and extracted with ether to remove any unreacted intermediate. The aqueous solution containing the sodium salts of the D- and L-ethers II (R = —CHMe.COOH) was carefully acidified to pH 4 with normal hydrochloric acid and the free acids (35 gm., 81 per cent) which were precipitated were filtered, washed with water and dried.

The mixed acids (32 gm.) were heated in refluxing benzene (200 ml.) for 15 min., the insoluble material removed by filtration and the filtrate allowed to stand at room temperature overnight before refiltering. The combined benzene-insoluble materials (10·6 gm.) were recrystallized from hot benzene, giving crystals, m.p. 208–210°, $[\alpha]_D^{20}$ − 26° (c, 2 in chloroform); infra-red absorption 1,635 cm.$^{-1}$ (C = N stretching vibration) and 1,705 cm.$^{-1}$ (C = O vibration), and analytical figures agreeing with those for the ether II (R = —CHMe—COOH). Acid hydrolysis in refluxing 2·7 N hydrochloric acid for 4 hr. followed by chromatography in phenol–water, 3 : 1 (w/w), revealed a ninhydrin-positive spot with R_F = 0·46 corresponding to the isomer of muramic acid[2].

The benzene solution of the soluble acids (21·4 gm.) was evaporated to 100 ml. and on standing at room temperature needle crystals (13 gm., m.p. approx. 160°) separated. Most of this material dissolved readily in warm ethyl acetate (5 ml./gm.) and filtration removed some of the isomer of m.p. 208–210°. On cooling and seeding, large needle crystals (5 gm.) separated. These had m.p. 163–164°; $[\alpha]_D^{20}$ + 65° (c, 2 in chloroform); infra-red absorption 1,635 cm.$^{-1}$ (C = N stretching) and 1,725 cm.$^{-1}$ (C = O vibration) and analytical figures agreeing with those for the ether II (R = —CHMe—COOH). Hydrolysis and chromatography as above, gave one spot R_F = 0·55 corresponding to muramic acid obtained from natural sources.

The acids remaining in the mother liquors were converted to the S-benzylthiouronium salts and recrystallization of the product from 20 per cent methanol in water gave 8 gm. of salt, m.p. 134–136°. On decomposition this gave 5·1 gm. of the acid m.p. 163–164°. (The S-benzylthiouronium salt of the acid m.p. 208–210° has not been obtained in the crystalline state.)

The acid of m.p. 163–164° (10 gm.) was hydrolysed in refluxing 2·7 N hydrochloric acid for 4 hr., the benzoic acid removed from the cooled hydrolysate by ether extraction and the hydrochloric acid removed by evaporation under reduced pressure. The residue was dissolved in water and 'Amberlite IR–4B' (OH form) added until the solution was at pH 6. After filtration, the aqueous solution was evaporated to dryness and the crystalline product (6·4 gm., 90 per cent based on muramic acid monohydrate) was recrystallized from water/ethanol. The product m.p. 150–151° (decomp.); $[\alpha]_D^{22}$ + 144° (4 min.) falling to + 112° (23 hr.) (c, 2 in water) gave satisfactory analytical figures for anhydrous muramic acid after drying for 6 hr. at 60° *in vacuo* over phosphorus pentoxide.

By a similar procedure, the carboxymethyl ether II (R = —CH_2.COOH) was prepared by the action of ethyl bromoacetate on the sodio derivative of the intermediate II (R = H). This had the following characteristics: m.p. 185–186° (from ethyl acetate); $[\alpha]_D^{19}$ − 22° (c, 2 in chloroform); infra-red absorption 1,635 cm.$^{-1}$ (C = N) stretching), and 1,725 cm.$^{-1}$ (C = O). The compound was hydrolysed and the product worked up as for muramic acid. The 3-O-carboxymethyl-D-glucosamine was recrystallized from water/ethanol, giving needle crystals m.p. 163–164° (decomp.); $[\alpha]_D^{22}$ + 130° (6 min.) falling to + 102° (24 hr.) (c, 2 in water). After drying for 6 hr. at 60° *in vacuo* over phosphorus pentoxide satisfactory analytical figures for the anhydrous material were obtained.

The reaction of methyl iodide with the sodio derivative of the intermediate gave the methyl ether II (R = —CH_3) in 90 per cent yield; m.p. 117–119° (from methanol); $[\alpha]_D^{20}$ − 15·5° (c, 2 in chloroform); infra-red absorption 1,647 cm.$^{-1}$ (C = N vibration). This compound was hydrolysed as above and the 3-O-methyl-D-glucosamine hydrochloride recrystallized twice from methanol/acetone; $[\alpha]_D^{22}$ + 112° (4 min.) falling to + 90° (18 hr.) (c, 2 in water).

Satisfactory analytical figures were obtained for all compounds described.

Roy Gigg

National Institute for Medical Research,
Mill Hill, N.W.7.

Patricia M. Carroll

Human Nutrition Research Unit,
Mill Hill, N.W.7.

[1] Jeanloz, R. W., *Adv. in Carb. Chem.*, **13**, 189 (1958).
[2] Strange, R. E., and Kent, L. H., *Biochem. J.*, **71**, 333 (1959).
[3] Zilliken, F., *Fed. Proc.*, **18**, 966 (1959).
[4] Lambert, R., and Zilliken, F., *Chem. Ber.*, **93**, 2915 (1960).
[5] Neuberger, A., *J. Chem. Soc.*, 50 (1941).
[6] Konstas, S., Photaki, I., and Zervas, L., *Chem. Ber.*, **92**, 1288 (1959).
[7] Kuhn, R., Zilliken, F., and Gauhe, A., *Chem. Ber.*, **86**, 466 (1953).
[8] Roth, W., and Pigman, W., *J. Amer. Chem. Soc.*, **82**, 4608 (1960).

Differential Inhibition of Human Serum Cholinesterase with Fluoride: Recognition of Two New Phenotypes

It has been shown that individuals excessively sensitive to the drug suxamethonium have a reduced level of serum cholinesterase activity, and that this peculiarity is genetically determined[1]. It has also been found that the cholinesterase present in the serum of such suxamethonium-sensitive individuals is qualitatively different from that occurring in most normal people[2]. This difference can be most simply demonstrated by determining the degree of inhibition produced by certain anticholinesterase substances. For example, using 5×10^{-5} M benzoylcholine as substrate and 10^{-5} M dibucaine as inhibitor under standard conditions, the enzyme in most normal sera shows about 80 per cent inhibition, while that in sera from suxamethonium-sensitive individuals shows only about 20 per cent inhibition. The percentage inhibition obtained under these conditions has been called the dibucaine number (D.N.) and this appears to be a constant characteristic for any one individual[3].

If sera from a large random sample of individuals in the general population are examined, the distribution of D.N. obtained is sharply trimodal, and people can be classified into three phenotypes on this basis[4]. About 97 per cent of the population have the so-called 'usual' phenotype with D.N. clustering around 80, about 3 per cent are of the so-called 'intermediate' phenotype with D.N. clustering around 62, and about

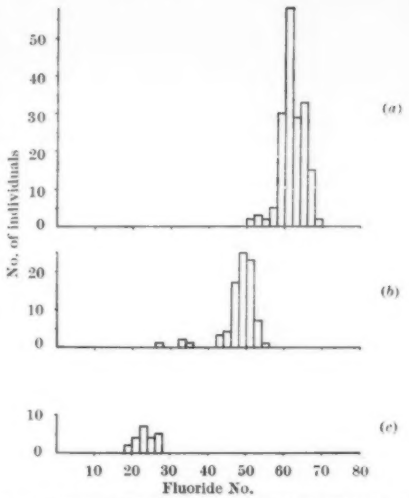


Fig. 1. Distributions of 'fluoride numbers' in individuals of the (*a*) 'usual', (*b*) 'intermediate' and (*c*) 'atypical' phenotypes as defined by dibucaine number determination

1 in 4,000 have the so-called 'atypical' phenotype with D.N. around 20. Suxamethonium-sensitive individuals belong to the 'atypical' phenotype. The three phenotypes are genetically determined. Two allelic genes are thought to be concerned, the 'usual' or normal phenotype being homozygous for one of these, the 'atypical' phenotype being homozygous for the other, and the 'intermediate' phenotype representing the heterozygotes[4,5]. It is likely that in the heterozygotes where both genes are present, both the 'usual' and the 'atypical' forms of the enzyme are synthesized in roughly equal amounts.

Kalow and Davies[6] have shown that a wide variety of inhibitors, but each containing a quaternary ammonium grouping, can be used to demonstrate this differential inhibition of the two enzymes. On the other hand, the differential inhibition was not obtained with the organophosphate inhibitors such as tetraethylpyrophosphonate (TEPP) or di*iso*-propylfluorophosphonate (DFP). They concluded that the esteratic site on the enzyme, where the latter inhibitors are believed to act, was probably the same in the two enzymes, while the anionic site where the positive charged quaternary ammonium inhibitors are thought to be attached is probably different.

We have now found, however, that differential inhibition of the enzymes present in the three phenotypes can also be obtained using sodium fluoride as inhibitor. Furthermore, the use of this inhibitor has enabled us to demonstrate the occurrence of at least two new phenotypes.

Sera from a series of individuals already classified by dibucaine number determinations were examined using fluoride as inhibitor. Inhibition with fluoride and determination of the so-called fluoride number (F.N.) were carried out in exactly the same way as dibucaine number determination except that 5×10^{-5} *M* fluoride was substituted for 10^{-5} *M* dibucaine in the test system. In all, sera from 179 individuals with the 'usual' phenotype, 84 individuals with the 'intermediate' phenotype, and 22 individuals with the 'atypical' phenotype were examined.

Fig. 1 shows the distribution of the fluoride numbers for the three classes. The means and standard deviations are given in Table 1. The F.N. obtained for the 'atypical' phenotype as defined by the D.N. determination are much lower than those for the 'usual' phenotype, and the phenotype 'intermediate' by D.N. determination is also intermediate in F.N. The discrimination between the three phenotypes using fluoride is nearly as clear cut as with dibucaine, and it is apparent that with few exceptions the classification of individuals obtained with fluoride is much the same as that obtained with dibucaine.

Table 1

Phenotype	No. of individuals	'Dibucaine number'		'Fluoride number'	
		Mean	Standard deviation	Mean	Standard deviation
'Usual'	179	80·06	1·56	61·35	3·21
'Intermediate'	84	61·93	4·17	47·79	4·48
'Atypical'	22	21·82	2·92	23·14	2·23

Table 2

Parental types	No. of families	Children			
		U	U_1	*I*	I_1
$U \times I$	20	36	—	32	—
$U \times I_1$	2	—	5	5	—

The exceptions, however, turn out to be of special interest. A plot of dibucaine number against fluoride number in the 285 individuals examined (Fig. 2) revealed that there was apparently a small sub-group of the 'intermediate' phenotype (D.N. 52–54, F.N. 26–34) in which the fluoride inhibition was relatively less marked than the dibucaine inhibition, and that a similar sub-group of the 'usual' phenotype also existed (D.N. 73–78, F.N. 50–55). The reality of these sub-groups has been confirmed by family studies. Thus, if we call the main groups of the 'usual' and 'intermediate' phenotypes *U* and *I* respectively, and the corresponding sub-groups U_1 and I_1, we have been able to compare the offspring from a series of matings $U \times I$ with those from $U \times I_1$ matings. The results are given in Table 2, and they indicate that U_1 and I_1 are genetically related.

These findings, and those from other types of mating, suggest that there may be a third gene which can influence the character of the cholinesterase

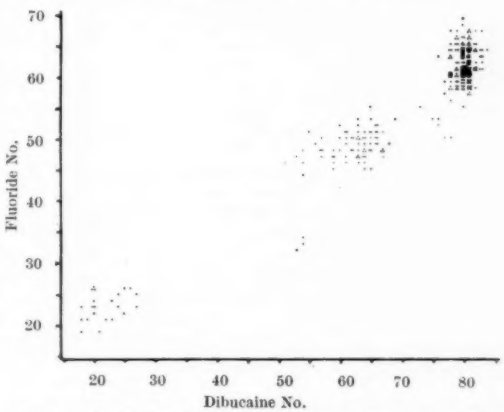


Fig. 2. Correlation between dibucaine numbers and fluoride numbers in the individuals shown in Fig. 1

synthesized. If so, the phenotype sub-groups, U_1 and I_1, would be determined by the presence of this gene in heterozygous combination, with the normal and abnormal genes previously identified. Possibly, this new gene determines yet a further variant of the enzyme which is qualitatively distinct, but much more work is required before this hypothesis can be substantiated.

We wish to thank Dr. H. Lehmann for many of the serum samples. We also wish to acknowledge a grant from the Central Research Fund of the University of London for the purchase of a spectrophotometer.

H. Harris

Department of Biochemistry,
King's College, University of London,
Strand, W.C.2.

Mary Whittaker

Department of Biochemistry,
The London Hospital Medical College,
London, E.1.

[1] Lehmann, H., and Ryan, E., *Lancet*, ii, 124 (1956).
[2] Kalow, W., *CIBA Foundation Symposium on Biochemistry of Human Genetics* (1959).
[3] Kalow, W., and Genest, K., *Canad. J. Biochem. Physiol.*, **35**, 339 (1957).
[4] Kalow, W., and Staron, N., *Canad. J. Biochem. Physiol.*, **35**, 1305 (1957).
[5] Harris, H., Whittaker, M., Lehmann, H., and Silk, E., *Acta Genet.*, **10**, 1 (1960).
[6] Kalow, W., and Davies, R. O., *Biochem. Pharmacol.*, **1**, 183 (1958).

The First Anthocyanins appearing during the Ripening of Blueberries

Although a great deal is known of the anthocyanins of berries and other fruit, this knowledge is still quite superficial and fragmentary[1]. How and in what order the anthocyanins develop in the maturing berries has only been clarified in one case: the first anthocyanin compound to appear in Californian 'Cabernet' grapes was found to be the malvidin-3,5-diglucoside; with advanced ripening of the grapes, malvidin-3-glucoside and delphinidin-3,5-diglucoside develop at almost the same time, while petunidin-3,5-diglucoside is the last to be formed[2]. Free malvidin was not observed in the grape skins until one week after the berries had developed the purple-red colour of complete maturity. These observations are noteworthy because they show that anthocyanins and not anthocyanidins are the first to appear during maturation. Furthermore, the first anthocyanin formed, malvidin-3,5-diglucoside, has quite a complicated structure. This anthocyanin is considered to be the dominant pigment in these grapes.

In order to gain further insight into the development of anthocyanins in maturing fruit, we decided to investigate the blueberry, *Vaccinium myrtillus* L., the characteristic berry of the northern forests of Europe. It is known that the ripe blueberry contains at least seven anthocyanins, derivatives of delphinidin, malvidin, petunidin and cyanidin[1]. Collecting the pigment from raw, reddish berries in sufficient amounts for identification was found to be too laborious a task. The pigments separated from raw berries were therefore compared with pigment fractions from ripe blueberries, which thereafter were used for the purpose of identification.

The pigments were extracted from the skins of raw, reddish blueberries with methanol containing 1 per cent hydrochloric acid. Two anthocyanins were found by paper chromatographic analysis of the methanolic extract (*n*-butanol/acetic acid/water, 4 : 1 : 5), one in amounts twice as large as the other. After acid hydrolysis and paper chromatographic analysis with 'Forestal' solution both these anthocyanins were identified as cyanidin derivatives. Free anthocyanidins were not found in the raw berries. Both anthocyanins were later examined by paper chromatography, with *n*-butanol/acetic acid/water (4 : 1 : 5), water/acetic acid/hydrochloric acid (82 : 15 : 3) and *n*-butanol/2 *N* hydrochloric acid (1 : 1) as solvent systems. The less-abundant compound was found to be identical with the third fraction, the more abundant pigment being identical with the fourth fraction obtained by column chromatography from ripe blueberries (Fig. 1).

The anthocyanins were extracted from the skins of ripe blueberries with the acidified methanol, precipitated as lead salts, dried, and again treated with the above solvent. From the regenerated solution a crude preparation of the pigment was obtained by addition of ether[2,3]. About 200 mgm. of the preparation was examined chromatographically

Table 1. R_F Values of the Aglycones of the Two Most Dominant Anthocyanin Fractions of Blueberries and of Some Cyanidin Preparations, in Different Solvent Systems

Anthocyanidin	AcOH/HCl/H_2O (30 : 3 : 10)	12 *N* AcOH/6 *N* HCl (2 : 1)	BuOH/2 *N* HCl (1 : 1)	BuOH/HCl/H_2O (7 : 2 : 5)	*m*-Cresol-12 *N* AcOH/6 *N* HCl (2 : 1/1)
Fraction 3	0·59	0·37	0·62	0·81	0·42
Fraction 4	0·50	0·37	0·62	0·81	0·42
Cyanidin (from red currants, *Ribes rubrum* L.)	0·50	0·35	0·60	0·81	0·42
Cyanidin (from cyanidin-3,5-diglucoside)	0·50	—	0·62	—	—

Table 2. R_F Values of the Two Dominant Anthocyanins of Blueberries and of Some Anthocyanin Preparations, in Different Solvent Systems

Preparation	BuOH/AcOH/H_2O (4 : 1 : 5)	BuOH/2 *N* HCl (1 : 1)	H_2O/AcOH/HCl (82 : 15 : 3)	BuOH/HCl/H_2O (7 : 2 : 5)
Fraction 3	0·38	0·26	0·22	0·43
Fraction 4	0·30	0·17	0·23	0·32
Cyanidin-3,5-diglucoside	0·15	0·05	0·38	0·19
Cyanidin-3-monoglucoside	0·30	0·17	0·24	0·33
Cyanidin-5-monoglucoside	colour unstable	0·38	0·07	0·57
Cyanidin-3-galactoside (from apples, *Pyrus malus* L.)	0·30	0·18	0·23	0·30
Cyanidin-3-galactoside (from rowanberries, *Sorbus aucuparia* L.)	0·32	0·16	0·23	0·30
Cyanidin-3-gentiobioside (from raspberries, *Rubus idaeus* L.)	0·24	0·16	0·59	0·39

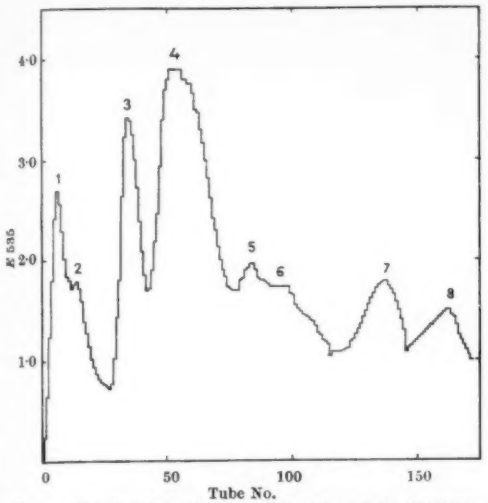


Fig. 1. Fractionation of the anthocyanins of the blueberry (*Vaccinium myrtillus* L.) using a cellulose column filled with Whatman cellulose powder and *n*-butanol/2 *N* hydrochloric acid (1 : 1) as developing solvent

in a column (5 cm. × 110 cm.) filled with cellulose powder (Whatman cellulose powder, standard grade or 'Linterspulver' No. 124, Schleicher and Schüll), *n*-butanol/2 *N* hydrochloric acid (1 : 1) being used as the solvent system[4]. The eluate was collected in 10-ml. fractions and the colour was measured at 535 mμ. From a graph with plots of extinction values (Fig. 1), it was found that the anthocyanins of the blueberry separate out into 8 peaks.

Samples of the eluates of peaks 3 and 4, which correspond to the anthocyanins of reddish blueberries, were hydrolysed by heating. The R_F values obtained by paper chromatography of the liberated anthocyanidins are shown in Table 1. The results establish that the aglycones of both anthocyanin fractions can be identified as cyanidin. This was confirmed by the distribution and oxidation tests and tests with the cyanidin reagent, sodium acetate and ferric chloride (Robinson's method).

The sugars were determined from the hydrolysed fractions 3 and 4. The water extracts were concentrated to about 10 ml., the hydrochloric acid was eliminated by extracting with 10 per cent di-*n*-octylmethylamine containing chloroform, and the solution was further concentrated to 1 ml. The sugars were identified by paper chromatography (*n*-butanol/acetic acid/water, 4 : 1 : 5), and it was observed that fraction 3 contained glucose and arabinose, fraction 4 glucose only. Control experiments proved that the arabinose was not an artefact produced either from the Whatman cellulose powder or from the paper (see ref. 5). Aniline–citric acid reagent was used in the quantitative determinations of the sugars. Comparison of the separately determined concentrations of the aglycones with the sugar concentrations proved that the concentration of glucose in both fractions was in almost equimolar proportion to the concentration of the anthocyanidin. The amount of arabinose, however, was found to be only half the equivalent amount, possibly owing to disintegration or partial splitting.

Table 2 shows the values obtained by paper chromatographic analysis of the anthocyanins. The R_F values of the anthocyanin in fraction 4, the largest fraction, in different solvent systems was found to correspond to the R_F values of cyanidin-3-glucoside. Considering the equivalent proportions of the aglycone and the sugar, the anthocyanin must be cyanidin-3-glucoside. In fraction 3 the R_F values of the arabinose-containing cyanidin derivative do not—as expected—agree with the R_F values of the reference preparations. According to the literature, however, the R_F values of this fraction closely approach the migration of cyanidin-3-glucopentosides.

In conclusion, the two anthocyanins initially appearing during the maturation process of blueberries are both found to be cyanidin derivatives. These two cyanidin derivatives, the first to appear being cyanidin-3-glucoside, and the arabinose-containing cyanidin derivative (possibly cyanidin arabinoglucoside), are the most abundant pigments of ripe blueberries, although it is known that the ripe berries also contain glycosides of malvidin, delphinidin and petunidin[1]. The order of appearance of these pigments during the ripening process bears out the earlier concept of Robinson[6] that biogenetically cyanidin should be regarded as the simplest of the anthocyanidins.

We thank Dr. C. G. Nordström for the preparation of cyanidin-3,5-diglucoside which he kindly placed at our disposal.

Heikki Suomalainen
A. J. A. Keränen

Research Laboratories of the
State Alcohol Monopoly (Alko),
Helsinki.

[1] Suomalainen, H., and Eriksson, Chr., *Lebensmittel-Untersuch. u. Forsch.*, **112**, 197 (1960).
[2] Bockian, A. H., Kepner, R. E., and Webb, A. D., *J. Agric. Food Chem.*, **3**, 695 (1955).
[3] Li, K. C., and Wagenknecht, A. C., *J. Amer. Chem. Soc.*, **78**, 979 (1956).
[4] Chandler, B. V., and Harper, K. A., *Nature*, **181**, 131 (1958).
[5] Harborne, J. B., *J. Chromatography*, **1**, 473 (1958).
[6] Robinson, R., *Nature*, **137**, 172 (1936).

PHYSIOLOGY

Effect of Administration of Lysergic Acid Diethylamide on Serotonin-Levels in the Body

In a previous publication[1], we have suggested that some of the effects of lysergic acid diethylamide (LSD) may be mediated through its effect on the serotonin content of brain. Brodie and his associates[2] have previously reported that LSD—and also some other drugs—do not release serotonin as reserpine does. However, Freedman[3] has recently reported slightly raised levels of serotonin in the whole brain of rats treated with LSD. Using animals which had been given reserpine, they found a twofold increase of brain serotonin in rats treated with LSD over the controls receiving reserpine alone. The whole brain serotonin may not be a true indication of the action of a psychopharmacological agent. Thus Vogt and her associates[4] have reported different effects of LSD and serotonin in different areas of the brain after intraventricular injection.

We have investigated the effect of LSD on the serotonin content of the different parts of the rabbit brain. The brain was divided into four major parts : (1) cerebral hemispheres, excluding midbrain, pons, thalamus, hypothalamus, etc., and mostly consisting

of the grey and white matter; (2) cerebellum; (3) stem; and (4) rest of the brain.

The animals were given intravenously 500 μgm. of LSD and were killed 15 min. later. In a second series of investigations, the rabbits were given intravenously 0·92 mgm., equal to 10 μc., of 5-hydroxytryptophan-3-^{14}C (5-HTP) followed by 500 μgm. of LSD 45 min. later. The experimental animals were killed 15 min. after the administration of LSD, while the controls received no LSD and were killed 1 hr. after the administration of the 5-HTP. Since the nature of the results as to the effect of LSD on the serotonin content was similar in both series of investigations, the results of the second series only are reported here. Table 1 presents the results as an average of four to six animals for each group. The first column represents the amount of total radioactivity per gm. of tissue and consists of the radioactivity due to 5-HTP, serotonin and their metabolites. The second column presents the content of serotonin determined spectrophotofluorimetrically, while the third one shows the specific radioactivity of the serotonin. The experimental details will be presented in detail elsewhere.

Table 1. Effect of Administration of Lysergic Acid Diethylamide on the Levels of Serotonin in the Body

Tissue	Total radioactivity (counts per min. per gm. tissue)		Serotonin (μgm. per gm. tissue)		Specific radioactivity of serotonin (counts/min./μgm.)	
	Control	LSD	Control	LSD	Control	LSD
Liver	884	665	1·74	2·62	14·3	15·0
Lung	545	439	1·02	1·46	7·0	3·0
Kidney	1,992	3,089	1·04	1·78	17·0	25·0
Spleen	1,753	1,713	42·14	55·14	1·8	2·3
Heart	277	346	1·34	3·86	—	3·3
Cerebrum	76	83	0·96	0·90	1·2	5·7
Cerebellum	112	450	1·24	1·72	0·7	2·1
Stem	108	273	2·12	2·94	—	6·8
Rest of brain	132	469	2·08	2·68	1·4	4·6

The effect of the administration of small quantities of 5-HTP was a small rise in the serotonin content of the tissues. The spleen is an extremely rich source of serotonin. Several interesting facets of serotonin metabolism are revealed by this work. The specific radioactivity of the serotonin is an index of the metabolic rate of serotonin in the tissue. Thus, kidney and liver are the most active sites, while the serotonin of the spleen is not in such an active state. It could be seen that the total radioactivity of the kidney is enhanced very much subsequent to the administration of LSD. This suggests that a considerably larger amount of the 5-HTP is transported to the kidney in the animal treated with LSD. The increase in the specific activity of serotonin is highest in the kidney, while on a percentage basis it is next highest to the brain tissues. This indicates an increased turnover of serotonin in the kidney and also a general increase in the serotonin content of the different parts of the viscera.

In the cerebrum there is no increase in the serotonin content in the animal treated with LSD. This is presumably due to the rapid destruction of the serotonin by oxidation, as is indicated by the higher specific activity of the serotonin. In other parts of the brain also, whereas the serotonin content increases only by approximately 40 per cent, the specific activity increases by about 350 per cent.

Thus these results indicate an overall increased metabolism of serotonin and an increase of the content of serotonin itself in all parts of the body examined, except in the cerebrum, subsequent to the administration of LSD. Whether the effects of LSD are specific or are shown by its non-hallucinogenic analogue, bromo lysergic acid diethylamide, and whether the action of LSD is limited to serotonin only without significant effects on other neurohormones such as noradrenaline is being investigated.

D. V. Siva Sankar
D. Barbara Sankar
Edward Phipps
Eleanor Gold

Biochemical Research Laboratory,
Children's Unit,
Creedmoor State Hospital,
Jamaica 27, New York.

[1] Siva Sankar, D. V., and Bender, L., in *Recent Progress in Biological Psychiatry*, edit. by Wortis, J., 363 (Grune and Stratton, New York, 1960).
[2] Carlsson, A., Shore, P. A., and Brodie, B., *J. Pharmacol. and Exp. Therap.*, **120**, 334 (1957).
[3] Freedman, D. X., *Fed. Proc.*, **19**, 266 (1960).
[4] Vogt, M., Gunn, C. G., and Sawyer, C. H., *Neurology*, **7**, 559 (1957).

Effect of the Bilateral Section of the Splanchnic Nerve on Erythropoiesis

Despite much recent work on erythropoietin which has established the important part played by the humoral factor in erythropoiesis, participation of the nervous system in the regulation of erythropoiesis has not been established. In view of the report of Jacobson *et al.*[1] suggesting the renal production of erythropoietin, as well as the work of Nakayama[2] that showed the effect of the bilateral section of splanchnic nerve in abolishing the decrease of renal blood-flow on loss of blood or anoxic anoxia, we performed a bilateral section of splanchnic nerve in rats to observe the effect on erythropoiesis.

The splanchnic nerve was sectioned bilaterally in the Wistar female rats (180–200 gm.) through the

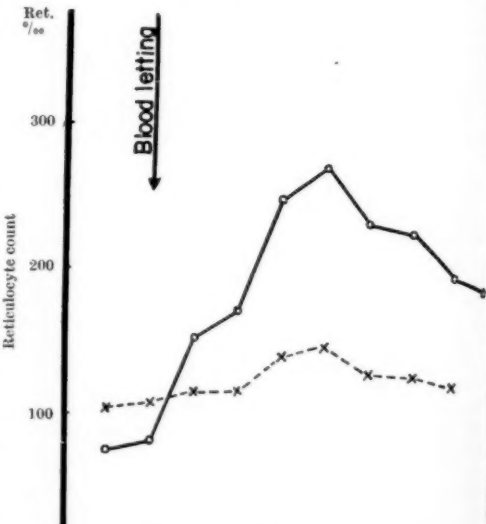


Fig. 1. Effect of bilateral section of splanchnic nerve on reticulocytosis after blood-letting (30 per cent of total blood volume). Each point represents the mean value for five rats. o, normal; ×, operated

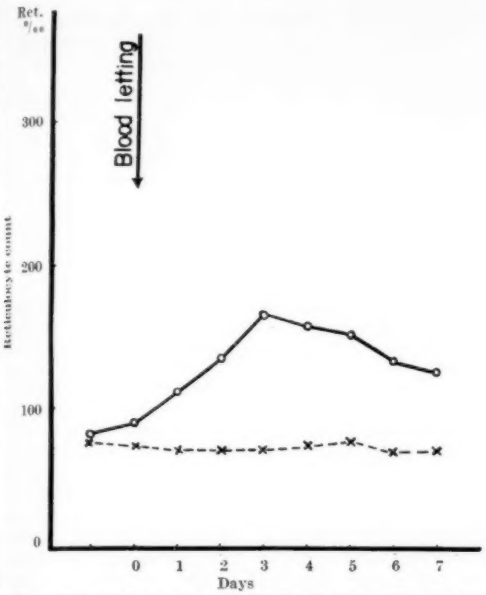


Fig. 2. Effect of bilateral section of splanchnic nerve on reticulocytosis after blood-letting (20 per cent of total blood volume). Each point represents the mean value of five rats. o, normal; ×, operated

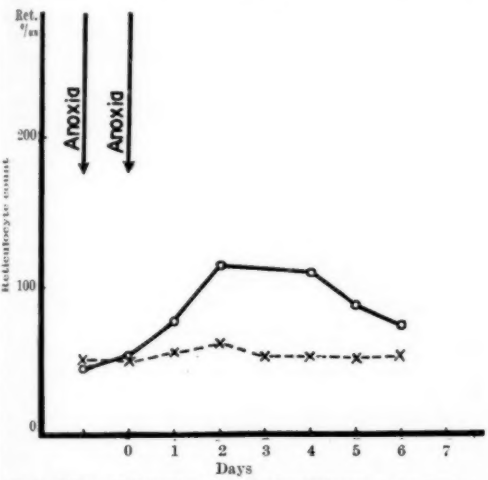


Fig. 3. Effect of bilateral section of splanchnic nerve on reticulocytosis after anoxic anoxia. Each point represents the mean value of five rats. o, normal; ×, operated

abdominal approach. After being bled as much as 1·0–1·5 per cent of their body-weight representing a loss of 20–30 per cent of the total blood volume[3] by cardiac puncture, the changes in the reticulocyte counts were followed in normal and operated rats. The results of experiments in Figs. 1 and 2 show that the loss of 30 per cent and 20 per cent of their total blood volume caused only a slight or no increase in reticulocytes in rats with a sectioned splanchnic nerve in contrast to the marked response to the same procedures for normal rats. When subjected to a mild anoxic anoxia, the rats with a sectioned splanchnic nerve showed no increase in their reticulocyte counts (Fig. 3). Such loss of the reticulocytic response in rats with sectioned splanchnic nerve suggests that the reduction of renal blood-flow which is regulated through the splanchnic nerve plays an important part in the production of erythropoietin when there is loss of blood or anoxic anoxia.

The reduction of the flow of arterial blood to the abdominal organs, including the kidney, in case of loss of blood has been explained as a mechanism for protecting the brain or heart from anoxia. By the experiments performed in our laboratory, it is suggested that the reduction of flow of arterial blood through the kidney in case of loss of blood occurs not only to protect the brain or heart but also to stimulate the erythropoietin production to facilitate the recovery from anæmia.

Fumimaro Takaku
Kunitake Hirashima
Shigeo Okinaka

Third Department of Internal Medicine,
School of Medicine,
University of Tokyo,
Bunkyo-ku, Hongo, Tokyo.

[1] Jacobson, L. O., Goldwasser, E., Fried, W., and Plzak, L., *Nature*, **179**, 633 (1957).
[2] Nakayama, S., *Respiration and Circulation*, **7**, 191 (1959) (in Japanese).
[3] Gray, S., and Sterling, K., *J. Clin. Invest.*, **29**, 1614 (1950).

Dependence on Substrate of the Electrical Potential across the Isolated Gut

Published reports[1,2] have indicated that fluid and salt transport across the isolated small intestine is very low in the absence of an external supply of substrate. Chemical measurements of net transport have shown that, of the many substrates tested, only glucose is able to support optimal absorption[1]. With such measurements, the tissue must be allowed to equilibrate for 20–30 min. before sufficient fluid or salt has been transported to allow accurate chemical measurements. Thus a considerable uncertainty exists as to how rapidly the absorptive process changes when glucose is added to or removed from the bathing media. A clarification of this point would indicate the extent to which utilizable energy is stored in the tissue.

By use of electrical techniques, rapid changes in salt absorption across the epithelia can be followed[3]. In this investigation, it was decided to monitor changes in the electrical potential difference established across the tissue as a consequence of the active absorption of sodium chloride[4,5]. Electrical potentials across isolated segments of rat small intestine (Rochester strain of Wistar) were measured by a technique described before[5], in which the small intestine could be transferred rapidly (within 3 sec.) from one solution bathing the mucosal side to another. Since the potential was found to be unaffected by the glucose concentration of the serosal solution, only the results of glucose changes in the mucosal solution will be presented.

Fig. 1 records the effects of glucose on the potential developed across the mid-jejunal segments. When the tissue was exposed to 200 mgm. per cent glucose in the presence of oxygen/carbon dioxide, the potential was maintained for at least 60 min. However, when the segment was transferred to a glucose-free medium a 60 per cent drop in potential occurred in 2 min. On returning to glucose medium, the potential was restored within 3–5 sec. These data clearly indicate that there is very little utilizable stored energy.

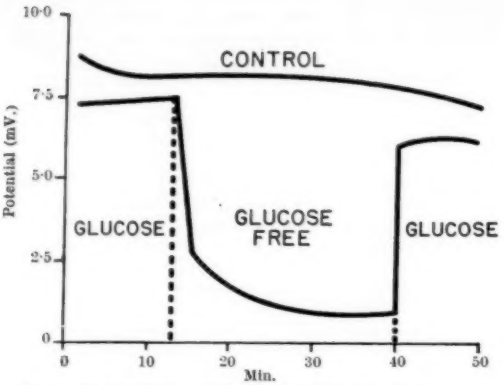


Fig. 1. Effect of glucose on the electrical potential across isolated segments of rat small intestine. Everted mid-jejunal segments were exposed to Krebs–Ringer bicarbonate buffer containing 200 mgm. per cent glucose and to a similar buffer containing no glucose at 37° C. in the presence of 95 per cent oxygen/5 per cent carbon dioxide. Ileal segments behaved identically except that their potentials were, on the average, 50 per cent less. The positive recording electrode was in contact with the serosal solution

The rapid recovery of the potential on addition of glucose deserves further comment. Chinard *et al.*[6] have reported that the mean transit time for passage of glucose across the mucosal cell of the proximal tubules of the kidney is 10 ± 3 sec. Since both cells have a similar structure and are able to transport glucose actively, one would conclude that the mean time of entry for glucose moving into the intestinal cell is about 5 sec., corresponding to the recovery time of the potential as noted in Fig. 1. Thus it is seen that the active transport of salt is stimulated immediately on the entry of glucose into the mucosal cell.

Investigations on the relationship of energy metabolism to active transport have been reported for frog skin[7] and toad bladder[8]. These epithelia have sufficient endogenous substrate to support active transport for many hours. On the other hand, the intestine, because of its complete dependence on external substrate, allows the person performing experiments a greater degree of control of the energy supply. For this reason the isolated small intestine presents an excellent preparation for the study of transfer of energy within the cell.

This investigation was carried out under contract with the United States Atomic Energy at the University of Rochester Atomic Energy Project, Rochester, New York.

T. W. Clarkson
A. C. Cross
S. Toole

University of Rochester School of Medicine,
Rochester 20, New York.

[1] Smyth, D. H., and Taylor, C. B., *J. Physiol.*, **136**, 632 (1957).
[2] Lifson, N., and Parsons, D. S., *Proc. Soc. Exp. Biol. Med.*, **95**, 532 (1957).
[3] Ussing, H. H., *Fourth International Congress of Biochemistry*, Symp. 3, 1 (Pergamon Press, London, 1959).
[4] Curran, P. F., and Soloman, A. K., *J. Gen. Physiol.*, **41**, 143 (1957).
[5] Clarkson, T. W., and Rothstein, A., *Amer. J. Physiol.*, **199**, 12 (1960).
[6] Chinard, F. P., Taylor, W. R., Nolan, M. F., and Enns, T., *Amer. J. Physiol.*, **196**, 535 (1959).
[7] Leaf, A., and Renshaw, A., *Biochem. J.*, **65**, 90 (1957).
[8] Leaf, A., Page, L. B., and Anderson, J., *J. Biol. Chem.*, **234**, 1625 (1959).

Soluble Material in the Gastro-intestinal Tract of Rats under Normal Feeding Conditions

Work on intestinal absorption has almost always been carried out under non-physiological conditions—on fasted animals fed by stomach tube, on anæsthetized animals or animals with fistulæ and on isolated loops. Moreover, single nutrients have usually been administered. It seemed desirable to obtain information under more nearly physiological conditions and to examine the medium from which absorption was proceeding at different levels of the gastro-intestinal tract.

Adult male rats were kept in an automatically lighted room arranged so that the period of darkness during which they do most of their feeding was 5 a.m.–2 p.m. They were provided with Thomson cube diet containing 14 per cent dried milk and water *ad libitum*. The animals were killed between 9·0 and 10·0 a.m. and immediately taken to a cold room (4° C.) where the stomach and small intestine were removed. The stomach (S) was tied off and the intestine divided into four arbitrary segments of approximately equal length labelled I_1–I_4, beginning at the pylorus. The contents of the stomach and intestinal segments were removed and spun in a refrigerated centrifuge at 4,000 r.p.m. for 20 min. This usually gave 0·05–0·5 ml. fluid for analysis.

In all animals the stomach contained 3–5 ml. of thick pasty material while the most proximal intestinal segment (I_1) was almost empty, containing only a small amount of thick mucous fluid with few insoluble food residues. The other intestinal segments (I_2–I_4) contained large amounts of material looking like homogenized rat cubes.

As shown in Table 1, there was a large reduction in the concentration of potassium between (S) and (I_2), the concentration in I_3 and I_4 remaining steady. The fall in the concentration of sodium was less pronounced. Chloride estimations were carried out by the Conway micro-diffusion method but the results were not included in the table since they varied widely from animal to animal. But while concentrations of more than 200 m.equiv./l. were sometimes found in the stomach the values for I_3 and I_4 never exceeded 10 m.equiv./l. The nature of the anions present in the lower half of the small intestine, where the concentration of $Na^+ + K^+$ amounted to 80–100 m.equiv./l., is not clear. Bicarbonate may have been present but was not estimated; yet supposing it was present at a level similar to that of plasma, that is, 27 m.equiv./l., this would leave more than 40 m.equiv./l. anions not accounted for. Some of the base may have been bound by the products of protein digestion since the amino nitrogen present in I_3 and I_4 was about 1 gm./l. or 70 m.equiv./l.

The concentration of total nitrogen in the centrifugates was highest in I_1, exceeding that in the stomach, and it fell progressively in the lower segments. The bulk of the nitrogen-containing material was not precipitable by sodium tungstate, that is, was non-protein nitrogen. The pattern for total nitrogen concentration was roughly paralleled by the non-protein levels.

Reducing and total sugar concentrations are expressed as glucose. There was a marked fall in the concentration of free and total reducing sugar between S and I_1 and this did not appear to be due to simple dilution since in all cases only small quantities of material were present in I_1, reflecting the rapid

Table 1. CONCENTRATION OF VARIOUS CONSTITUENTS OF THE SOLUBLE MATERIAL PRESENT AT DIFFERENT LEVELS OF THE GASTRO-INTESTINAL TRACT OF RATS UNDER NORMAL FEEDING CONDITIONS

Segment	S	I_1	I_2	I_3	I_4
Potassium (m.equiv./l.) Sodium (m.equiv./l.)	135 (± 9·3) 136·6 (± 9·9)	51·5 (± 6·6) 109·4 (± 14)	29·0 (± 3·0) 85·1 (± 18)	18·5 (± 3·5) 80·1 (± 8·7)	18·6 (± 1·9) 68·3 (± 10·0)
Total nitrogen (gm./l.) Non-protein nitrogen (gm./l.)	6·6 (± 1·0) 4·16 (± 1·3)	9·1 (± 3·1) 6·56 (± 3·1)	6·3 (± 1·3) 5·8 (± 1·5)	4·77 (± 0·45) 4·02 (± 0·6)	3·85 (± 0·26) 3·44 (± 0·28)
Free reducing sugar (as gm. glucose/l.) Total reducing sugar (as gm. glucose/l.)	68·4 (± 5·8) 93·4 (± 15·4)*	10·9 (± 3·6) See footnote†	21·2 (± 3·8) 29·3 (± 5·6)*	21·5 (± 2·9) 33·6 (± 4·7)*	20·8 (± 4·1) 35·1 (± 6·2)*

Figures in brackets show the standard deviation for five observations except where indicated by *, when only four observations were made.
† Three observations only. In these, the increase in reducing power on hydrolysis was 1·61 (± 0·29) gm./l.

passage of food through the upper part of the small intestine. The slight difference between the free and total reducing sugar in this segment suggested that monosaccharides constituted the major carbohydrate fraction.

The free reducing sugar was remarkably constant between segments I_2, I_3 and I_4, where it was present at a concentration of about 2 per cent. This constancy was unexpected since it is generally believed that all digestible carbohydrate is absorbed before the chyme leaves the small intestine, and some decrease in reducing power in the last segment might therefore have been expected. The results are of interest in view of Fisher's[1] finding that absorption of water only proceeds from the isolated small intestine of the rat when glucose is present in the lumen.

It is emphasized that these are preliminary results with a small number of animals (5) and that certain improvements of technique are considered to be necessary, for example, a different method of killing instead of the ether anæsthesia and bleeding used here; nevertheless the results are sufficiently consistent to suggest that this more physiological approach to the study of gastro-intestinal function may be of value.

ANNE S. COLE

Department of Physiology,
University of Bristol.

[1] Fisher, R. B., *J. Physiol.*, **130**, 655 (1955).

HÆMATOLOGY

Stability of Freeze-dried Anti-hæmophilic Globulin

THE stability of anti-hæmophilic globulin in solution has been the subject of many investigations. Hitherto, no systematic work on the factors affecting the activity of dried material has been reported.

In order to obtain comparable results in the present investigation, the period necessary for the loss of 50 per cent of the activity of anti-hæmophilic globulin[1], subjected to the necessary conditions of each experiment, was estimated. Bovine, porcine and human anti-hæmophilic globulins were utilized; no appreciable difference was observed in their behaviour.

Type of container: Anti-hæmophilic globulin stored at room temperature in stoppered M.R.C. bottles or universal containers lost half the activity in two months. Storage in darkness had no significant effect. On the other hand, sealed ampoules preserved the same activity for four months.

Effect of temperature: Sealed ampoules were used in this test. Deterioration was rapid at 37° C.; the standard loss occurred after 5–8 days. At 4° C. the same loss was observed after storage for six months.

Storage under vacuum over phosphorus pentoxide: This had practically the same effect as that produced by storage in sealed ampoules.

Humidity: When samples of anti-hæmophilic globulin in loosely stoppered universal bottles were kept in a humid atmosphere, two weeks were sufficient to lower the activity to 50 per cent.

Time of packing: The potencies indicated on commercial ampoules were invariably found to be higher than my estimates. This led to inquiries which showed that in certain cases this was most probably due to a time-lag between the completion of the drying process (when the activity was determined) and packing. When this delay was minimized, not only did our figures coincide but also the preservation of the activity was prolonged; the subsequent loss of activity of samples treated in this way was almost halved.

It would now appear that in order to obtain and preserve the maximal activity of anti-hæmophilic globulin, delay should be avoided in drying and sealing the material in evacuated or nitrogen-filled ampoules, which should be stored at 4° C.

This work was supported by a grant from the United Manchester Hospitals. I thank Dr. J. F. Wilkinson for laboratory facilities.

F. NOUR-ELDIN

Department of Pathology,
Southmead Hospital,
Bristol.

[1] Nour-Eldin, F., *Brit. Med. J.*, ii, 502 (1960). Nour-Eldin, F., and Wilkinson, J. F., *Brit. J. Hæmat.*, **4**, 292 (1958).

New *Rh* Phenotype, *Dcce^ie^if*, found in a Chibcha Indian Tribe

IN the course of serological work on various Chibcha-Indian tribes of Colombia it was found that the blood of 6 out of 113 Ica Indians, inhabitants of the Sierra Nevada of Santa Marta, reacted with anti-*D* and anti-*c* sera but failed to react with anti-*C*, anti-*E*, and anti-*e* sera. Further laboratory work carried out at the Caracas Blood Bank as well as in the Blood Group Research Unit of the Lister Institute of London and at the Mount Sinai Hospital in New York has demonstrated that the six samples previously mentioned reacted with anti-*f* and with some anti-*e* sera as well.

Considering these results, we may call the new phenotype *Dcce^ie^if* and the chromosome *Dce^if*(R_i), using the *i* as indicative of the tribal denomination of the Ica among whom the phenotype was found for the first time. Genetic studies of a family carrying this chromosome, together with the serological evidence for the differentiation of this phenotype from other *Rh* gene complexes like *Dc*- (ref. 1), will be published elsewhere (Layrisse, M., Layrisse, Z., and Wilbert, J.).

Table 1 shows the frequencies of the *Rh* phenotypes and chromosomes found in 113 samples. For the calculation of the chromosomes we assumed that the phenotype *Dcce* is the expression of *dce*/*Dce*^{i}, since the chromosome *Dce* is rare in pure Indians and since no Negro genes were found in this population: *Js*(*a*–), hæmoglobins *S* and *C* were negative, and the gene *B* (0·44 per cent) was lower than A_1 (1·34 per cent).

Table 1. *Rh* Frequency in Ica Tribe using Anti-*D*, Anti-*C*, Anti-*c*, Anti-*E* and anti-*e*

Phenotypes	Genotypes	No. observed	Frequency observed	Frequency expected	$\left(\frac{\text{Obs.}-\text{exp.}}{\text{Exp.}}\right)^2$
DCCEE	*DCE*/*DCE*	0·00	0·00	0·00	—
DCCEe	*DCE*/*DCe*	0·00	0·00	0·00	—
DCCee	*DCe*/*DCe*	46·00	40·71	36·75	0·43
DCcE	*DCE*/*Dce*^{i}	0·00	0·00	0·00	—
	DCE/*DcE*				
DCcEe	*DCe*/*DcE*	10·00	8·85	13·39	1·53
DCce	*DCe*/*dce*	35·00	30·97	34·35	0·33
	DCe/*Dce*^{i}				
DccE	*DcE*/*DcE*	5·00	4·42	5·66	0·25
	DcE/*Dce*^{i}				
DccEe	*DcE*/*Dce*	5·00	4·42	1·81	0·44
Dcce	*dce*/*Dce*^{i}	5·00	4·42	3·31	
ddccee	*dce*/*dce*	1·00	0·89	0·68	
Dcce^i*ei*	*Dce*^{i}/*Dce*^{i}	6·00	5·31	4·04	0·40
Total		113·00	99·99	99·99	3·38

$0·70 > P > 0·50^{n=5}$

Chromosomes (per cent)

DCe	60·62
DcE	11·05
dce	8·22
Dce^i	20·11

The peculiar distribution of the chromosome *Dce*^{i}*f* among the Chibcha tribes studied is rather surprising. Whereas it occurs in a relatively high frequency among the Ica (20 per cent), the phenotype which identified this chromosome was absent in the two other tribes (Tunebo and Paez) of the same linguistic affiliation (Layrisse, M., Layrisse, Z., and Wilbert, J.).

Since not more than 10 South American Indian tribes have been tested with both anti-*E* and anti-*e*, we shall have to wait for further results before any statement concerning the frequency of the new chromosome in the autochthonous population of the New World can be advanced.

We thank Drs. R. Sanger and R. R. Race for assisting in the study of the family carrying the chromosome; Dr. R. E. Rosenfeld, who provided anti-*f* serum and tested one case of the phenotype *Dcce*^{i}*f* shown in Table 1, and Dr. Levine, who provided another serum used in this work. The Creole Foundation, Caracas, sponsored the project and the Instituto Colombiano de Antropología rendered most useful assistance.

Miguel Layrisse
Zulay Layrisse
Esperanza García

Instituto Venezolano,
Investigaciones Cientificas,
Centro de Investigaciones,
Banco Municipal de Sangre,
Caracas, Venezuela.

Johannes Wilbert

Fundación La Salle de
Ciencias Naturales,
Caracas, Venezuela.

Joaquín Parra R.

Instituto Colombiano de
Antropología.
Bogotá, Colombia.

[1] Tate, H., Cuningham, C., McDade, M. C., Tipett, P. A., and Sanger, R., *Vox Sanguinis*, **5**, 398 (1960).

HISTOCHEMISTRY

Direct Relationship of Phosphorylase and Mitochondrial α-Glycerophosphate Dehydrogenase Activity in Skeletal Muscle

Previous work[1] showed that in mammalian and avian muscle fibres there was a reciprocal relationship between phosphorylase and the oxidative enzymes, as shown by histochemical techniques. Two groups of fibres were described. In the histologically larger group, there was high phosphorylase activity and low activity of oxidative enzymes. (These included succinate, lactate, malate, isocitrate, β-hydroxybutyrate, alcohol and glutamate dehydrogenases, and also the DPN-linked α-glycerophosphate dehydrogenase.) In the group of histologically smaller fibres the converse activities were present.

With the development of a histochemical technique capable of demonstrating the purely mitochondrial α-glycerophosphate dehydrogenase[2], which is not linked with coenzyme, its localization in muscle has become possible. The enzyme is found to be strongly concentrated in the group of larger fibres. Activity in the smaller fibres is weak or absent. Mitochondrial α-glycerophosphate dehydrogenase activity thus runs parallel with phosphorylase activity, unlike all other oxidative enzyme systems so far tested.

Since the non-mitochondrial α-glycerophosphate dehydrogenase is strongest in the group of smaller fibres, the present findings do not fully support the concept that an α-glycerophosphate cycle[3] is an important metabolic pathway in the larger fibres. They do, however, fit the supposition that dihydroxyacetone phosphate, produced by α-glycerophosphate dehydrogenase activity, could enter the Embden-Meyerhof pathway at the level of triosephosphate isomerase. They provide further support for the thesis that the two types of muscle fibre rely substantially on different metabolic pathways in obtaining their supplies of energy for muscular contraction.

A. G. E. Pearse

Postgraduate Medical School,
London, W.12.

[1] Dubowitz, V., and Pearse, A. G. E., *Nature*, **185**, 701 (1960); *Histochemie*, **2**, 105 (1960).
[2] Wattenberg, L. W., and Leong, J. L., *J. Histochem. Cytochem.*, **8**, 296 (1960). Hess, R., and Pearse, A. G. E., *Nature* (in the press).
[3] Bücher, Th., and Klingenberg, M., *Angew. Chem.*, **70**, 552 (1958). Zebe, E., Delbrück, A., and Bücher, Th., *Biochem. Z.*, **331**, 254 (1959).

HISTOLOGY

Deoxyribonucleic Acid Content of Nuclei dividing amitotically

The presence of a rather high percentage of binucleated cells in the rat epididymis, especially in the body and tail, is well known[1–3]. The two nuclei, which are approximately equal in volume, arise by amitotic division, as judged by the complete series of morphological stages which can be traced from a small radial fold in the nucleus to its complete subdivision by a furrow. The nuclei remain in close contact with one another, usually with their apposed surfaces flattened.

It is now known that cells about to divide mitotically have twice the deoxyribonucleic acid (DNA) content of resting, undividing cells and that binucleate

cells, at least in the liver, have twice the normal diploid amount of DNA[4–6]. It seemed of interest to determine whether the same phenomena would be observed in cells dividing amitotically.

Small portions of the neck of the rat epididymis were dissociated by trypsin digestion and squashed in isotonic sucrose. Fixation for 15 min. in formalin vapour was followed by extraction of plasmalogenic lipids in alcohol and boiling alcohol-ether. After rehydration the slides were stained by the Feulgen procedure. The nuclei were homogeneous and well suited to photometry.

The optical density of the nuclei of mononucleate and binucleate cells was determined in a modified Lison-type histophotometer. With the densities encountered glare could be completely neglected. The projected area of each nucleus was sampled representatively (10 readings) and the resulting mean density multiplied by the area (planimetrically determined) to give DNA content in arbitrary units. Since there was some variation in the measured mean DNA contents in the five specimens of epididymis examined, the values were recalculated to a common mean on the basis of the DNA content of the mononucleate cells. These always formed a single population of nuclei as judged by inspection of the frequency histograms.

Histograms showing the DNA contents of both mononucleate and binucleate cells are shown in Fig. 1. It will be seen that the nuclei of mononucleate cells form a single population. Inspection shows no evidence of any skewness of the frequency distribution, as might be expected if there were a doubling of DNA content before the amitosis.

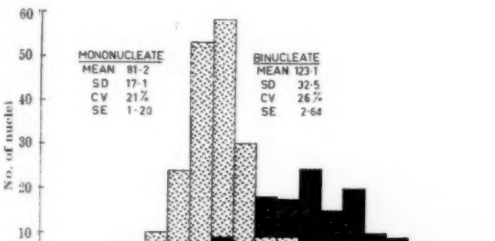


Fig. 1

The binucleate cells also form a single population of cells with respect to DNA content, the mean value being almost exactly half as much again as that of the mononucleate cells ($123{\cdot}1 \pm 2{\cdot}64$ as against $81{\cdot}2 \pm 1{\cdot}20$).

It is clear that there is no doubling of the DNA content of the nuclei either before or after amitotis but that, when the amitosis has occurred, there is an increase in DNA content to a value which is 150 per cent of that of the mononucleate cells.

These results can be explained by the following theory: in a nucleus like a rat epididymal nucleus which is not subject to mitosis or endomitosis (endopolyploidy) the presence of two homologous chromosomes normally inhibits duplication of either one. When, however, the homologous chromosomes are separated from one another by the development of the nuclear partition which arises during amitotic division, the control mechanism no longer operates and each duplicates itself (polysomaty or polynemy).

When the chromosome number is large (as in the rat), and in the absence of any somatic pairing, the distribution of paired and unpaired chromosomes in the two nuclei arising by amitosis closely approximates a binomial distribution in which $p = q = 0{\cdot}5$ and $n = 21$ (the number of chromosome pairs). The mean value is 10·5 paired and 21 unpaired chromosomes. Duplication of these unpaired chromosomes would lead to an increase of 50 per cent in the DNA content of the two nuclei.

The total variability in the binucleate cells is the result of normal biological and experimental variation and of the variation in the number of paired chromosomes. The first source may be calculated from the variance found in mononucleate cells and the second calculated from the above binomial expression. On this basis the expected standard deviation of the DNA content of the binucleate cells is 32, which is very similar to the value actually obtained (32·5).

The work here described appears to conflict with that of Vendrely[7], who measured the DNA content of about 90 nuclei chosen randomly from serial sections of mouse epididymis. Normal values were found except for some intermediate between diploid and tetraploid levels; these were ascribed to the presence of mitotic activity in the epididymis. Vendrely does not say how many of the nuclei measured were double.

I am indebted to Prof. H. O. Lancaster for help with the statistics. The work has been supported by the National Health and Medical Research Council and the Normal Haire and Cancer Research Funds of the University of Sydney.

K. W. Cleland

Department of Histology and Embryology,
University of Sydney.

[1] Regaud, C., *C.R. Soc. Biol., Paris*, **53**, 616 (1901).
[2] Benoit, J., *Arch. Anat. Strasbourg*, **5**, 174 (1926).
[3] Reid, B. L., and Cleland, K. W., *Austral. J. Zool.*, **5**, 223 (1957).
[4] Swift, H., *Internat. Rev. Cytol.*, **2**, 1 (1953).
[5] Vendrely, R., and Vendrely, C., *Internat. Rev. Cytol.*, **5**, 171 (1956).
[6] Leuchtenberger, C., in *Gen. Cytochem. Methods*, **1**, 220 (1958).
[7] Vendrely, C., *C.R. Soc. Biol., Paris*, **152**, 652 (1958).

Photo-Sensitivity of Thiazine Leucobases

During the course of experiments designed to investigate the staining of nucleic acid in histological sections, I happened to gather together on one bench a number of solutions of basic dyes reduced to their colourless sulphite leucobase. These included methyl green, basic fuchsin and a series of thiazines, namely, thionin, azure *A*, *B* and *C*, and methylene blue (for a discussion of the structure of these thiazine dyes, see Baker[1]).

The basic fuchsin leucobase (Schiff reagent) was prepared according to the directions of de Tomasi[2]. The other leucobases were prepared by dissolving 8 gm. of sodium thiosulphate in 100 c.c. of a 0·1 per cent solution of the dye in question. Once solution was effected, 0·5 c.c. concentrated hydrochloric acid was added, and the mixture heated over a bunsen flame for 30 sec. The result was a decolorized solution with a grey, curdy precipitate, best left unfiltered, as it appeared to stabilize the solution.

An accidental shaft of sunlight from a point source produced an intriguing result. Each of the thiazine leucobases developed a blue spot in the solution in

relation to the point source of sunlight. The blue spot disappeared a few seconds after the solution was shielded from light. The leucobases of methyl green and basic fuchsin showed no such phenomenon. Further examination showed it was not elicited by the light from an ordinary tungsten filament bulb, although the tungsten light source in my microscope, distinctly blue at top intensity, was effective. Daylight indoors had very little result, but daylight outside even with a cloudy sky produced the blue colour almost immediately.

Ultra-violet light seemed the most likely excitant, so the leucobase solutions were placed in front of a Wood's lamp (mercury discharge). Blue-green fluorescence was immediately observed, maximal in the case of methylene blue and the azures, moderate in the case of thionin, absent in methyl green and basic fuchsin leucobases. On subsequent examination, all the leucobases which had fluoresced were also showing photo-oxidation, as proved by the return of the original dye colour, and its subsequent disappearance in the dark.

It appears clear that leuco-thiazines show various effects when irradiated with ultra-violet and possibly visible blue light. Effects of this kind may be responsible for the reputation which these leucobases have for 'instability'. In fact, thiosulphate preparations of thiazines will remain stable for months if kept in a dark, well-stoppered bottle. Even in Coplin jars with loosely fitting lids, reduction will be maintained for almost two weeks, if care is taken to exclude light.

All the dyes described in this communication were supplied by 'Chroma' of West Germany.

D. W. Menzies

Department of Pathology,
University of Melbourne.

[1] Baker, J. R., *Principles of Biological Micro-Technique*, 180, 268 (Methuen and Co., London, 1958).
[2] de Tomasi, J. A., *Stain Technol.*, **11**, 137 (1936).

PATHOLOGY

Effects of *Grewia populifolia* Extracts on the Development of Chick Embryos

In the course of screening work with various plant extracts for their carcinolytic activities, *Grewia populifolia* extract was found to have anti-tumour activities when injected in rats bearing rhabdomyosarcoma (Kundu *et al.*, unpublished work). It was decided to inject this extract into fertile eggs to study its effects on the organogeny of early chick embryos in a way similar to that of Jurand[1].

Four different concentrations, namely, 16×10^{-5}, 20×10^{-5}, 24×10^{-5} and 32×10^{-5} gm. per c.c., were used. For each concentration, individual eggs from different groups received 1 c.c. of the solution; the *p*H of these varied from 6 to 7. It was found difficult to inject 1 c.c. of the solution into the egg. To overcome this, 1 c.c. of albumen was taken out with a syringe and replaced by the same volume of normal saline solution containing the required concentration. The injection was made quite near the blastoderm through a small hole that was resealed and covered with 'Cellotape'.

Before the injection, each group was incubated at 38° C. for nearly 18 hr. After injection, this was kept at 25° C. overnight to allow the extract to mix with the albumen, and then re-incubated for periods of 24 hr. and 48 hr. respectively. The controls were treated similarly and they received 1 c.c. of normal saline after the removal of 1 c.c. of albumen.

Fixation was carried out with acetic acid-alcohol. Sections were cut at 10μ thickness and stained with celestine blue. In some cases eosin was used as the counter stain.

Of the four concentrations tested, 16×10^{-5}, 20×10^{-5} gm. per c.c. had no effect. This was revealed when the sections were examined under a microscope. A concentration of 32×10^{-5} gm. per c.c. appeared to be very toxic, as in all the eggs receiving this concentration the blastoderm at the time of inspection was disintegrated.

With 24×10^{-5} gm. per c.c. concentration, 60 per cent of the embryos were affected. The abnormalities observed were more or less similar in all the affected embryos. The most noticeable changes, observed after 48 hr. incubation, were the reductions in size of the embryo proper and the blastoderm. Examination of the sections revealed that the neural system was most severely affected throughout. The brain and the neural tube were enlarged, and their lumina were partially filled up with degenerated cell debris. In the brain particularly, the inner and the outer walls were broken, and the cells became loose with pycnotic nuclei (Fig. 1). Mitosis was rare. A section through the brain region from a control specimen clearly revealed the difference (Fig. 2).

In the neural tube, the outer wall was intact but the cell damage was similar to the brain tissue. The notochord revealed the same picture, but the sheath was intact. There was an increase in the mesenchymal cells and the dorsal aortæ were gorged with blood cells. The somites were affected only slightly in the posterior region of the embryo, and there was a clear differentiation into sclerotome, dermatome and myotome.

Examination of sections from embryos fixed after 24 hr. incubation showed that the neural tissue was enlarged. The inner wall of the brain and the neural tube were broken at places and cellular material

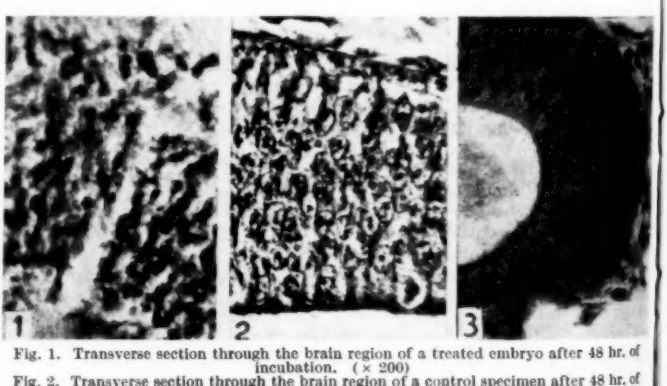


Fig. 1. Transverse section through the brain region of a treated embryo after 48 hr. of incubation. (× 200)
Fig. 2. Transverse section through the brain region of a control specimen after 48 hr. of incubation. (× 200)
Fig. 3. Transverse section through the brain region of a treated embryo after 24 hr. of incubation. (× 100)

was found scattered in the lumen, which appeared to have come from the cells lining it (Fig. 3). Mitosis could be observed, though less than the control. Other tissues which were observed to be affected after 48 hr. were more or less normal.

It appears from the present series of experiments that with concentrations of 24×10^{-5} gm. per c.c. of extracts of *G. populifolia* some organs of the developing embryos are affected. In the neural tissue there is an antero-posterior gradient in the degree of susceptibility, the brain being more affected than the neural tube, thereby showing that regions having a high rate of mitosis are more susceptible. This might also account for the lower degree of susceptibility of the somite where the rate of mitosis is not so great as in the two former tissues.

For the expansion of blastoderm tension is necessary, as reported by New[2]. This tension might have been reduced by breaking the intercellular connexions, resulting in a reduction of its size. A similar explanation has been given by Overton[3].

Engorgement of the blood vessels and an increase in the mesenchymal cells in the embryo proper might be due to the reasons suggested by Overton[3] and Waddington *et al.*[4].

The presence of mitosis after 24 hr. treatment and more or less its complete absence after 48 hr. with cellular derangement suggest that the extract of *G. populifolia* might have acted as a cytotoxic agent.

We are grateful to Dr. Subodh Mitra, director of this Centre, for his interest in the work, and to Dr. P. De for valuable discussions.

S. K. Brahma
N. Kundu

Department of Cell Research and Chemotherapy,
Chittaranjan National Cancer Research Centre,
37 S.P. Mookerji Road,
Calcutta 26.

[1] Jurand, A., *J. Embryol. Exp. Morph.*, **6**, 357 (1958).
[2] New, D. A. T., *J. Embryol. Exp. Morph.*, **7**, 146 (1959).
[3] Overton, J., *J. Exp. Zool.*, **139**, 329 (1958).
[4] Waddington, C. H., Feldman, M., and Perry, M. M., *Exp. Cell Res.*, Supp., **3**, 366 (1955).

Pathological Parthenogenesis in a Viviparous Toothcarp

In viviparous toothcarps parthenogenesis has been described in *Lebistes reticulatus*[1,2] and *Xiphophorus helleri*[2], while in this species also some cases of development of ovarial teratomas, caused by pathological parthenogenesis, are known[3]. In addition to these cases we observed a new one, namely in *Heterandria formosa*. It concerns a female isolated immediately after birth and consequently free from any contact with the male sex. After reaching a length of 27 mm. the virginal animal dropped 4 young fishes, all female. After this the animal became pregnant twice again, resulting in the birth of 6 and 10 young fishes, respectively, also exclusively female. For microscopic confirmation of the virginity the ovarial sperm chambers were examined, no spermia being observed. This finding ruled out self-fertilization, a phenomenon incidentally found in viviparous Cyprinodonts and explained by the presence of a bisexual gonad producing oocytes as well as spermia. As in the cases already described[2,3], an infection process caused by the Phycomycete *Ichthyophonus hoferi* was present in the cranial part of the ovary and in the adjacent regions. If an extensive process had been localized in the caudal parts of the ovary and near the junction between ovary and oviduct, birth would undoubtedly have been prevented. The young females appeared without an exception to be haploid.

The coincidence of the *Ichthyophonus* infection on one hand and the parthenogenetic development on the other justifies the assumption that the pathological process in this case had caused the parthenogenetic development. It could be assumed that the *Ichthyophonus* toxin provided the artificial stimulus for the development of the unfertilized oocytes.

Summarizing, we may conclude that this case forms an argument for my theory of pathological parthenogenesis[2,3]. It would certainly be advisable in any new cases of parthenogenesis to carry out a systematic examination for the possible presence of a pathological process in the ovary or in the immediate surroundings of this organ.

A. Stolk

Department of Histology,
Free University,
Amsterdam.

[1] Spurway, H., *Nature*, **171**, 437 (1953).
[2] Stolk, A., *Nature*, **181**, 1660 (1958).
[3] Stolk, A., thesis, Utrecht (1950); *Proc. Kon. Ned. Akad. Wet. Amsterdam, C* **56**, 28 (1953); *Nature*, **183**, 763 (1959).

Diagnostic Significance of Heterogeneous Lactic Dehydrogenases in Malignant Effusions

There is no laboratory test available for the differentiation of benign effusions from malignant exudates. Wroblewski and Wroblewski[1] reported in 1958 that, in malignancy, concentration of lactic dehydrogenase is frequently higher in effusions than in the corresponding sera. Nevertheless, the diagnostic value of these determinations is restricted since in about one-third of all cases investigated the amount of the lactic dehydrogenase in serum and effusion was the same[1]. Recent work on lactic dehydrogenase of tissues[2] and serum[3–5] indicates that this enzyme occurs in a number of molecular species which can be distinguished on account of their kinetics, antigenicity, inhibition by diphosphopyridine nucleotide analogues and electrophoretic mobility. In serum, starch block[3,4] and agar gel[5] electrophoresis and chromatography on diethylaminoethyl (*DEAE*) cellulose[6] showed the presence of at least four dehydrogenases, which are best labelled according to their electrophoretic mobility, namely, α_1, α_2, β and γ-lactic dehydrogenase. A specific increase in particular fractions has been observed in the following diseases: α_1 in myocardial infarction[3–6], $\alpha_1 + \alpha_2$ in muscular dystrophy[4] and γ in liver disease[5,6].

No information about the heterogeneity of lactic dehydrogenase in effusions is available. Since differences between malignant and benign effusions might occur, some thirty effusions were investigated by starch block electrophoretic separation[7] and determination of lactic dehydrogenase of each fraction using the optical test[8]. Effusions were centrifuged and the sediment discarded. 0·2 ml. serum or effusion was separated on starch block electrophoresis into four main fractions: α_1, α_2, β and γ. Lactic

Table 1. Lactic Dehydrogenase Iso-enzymes in Effusions (Starch Block Electrophoresis)

No.	Age	Sex	Diagnosis	Lactic dehydrogenase in serum					Lactic dehydrogenase in effusions				
				Total	α_1	α_2	β	γ	Total	α_1	α_2	β	γ
H-F, *H-D*	72	M	Carcinoma of œsophagus	4·0	1·2	2·1	0·5	0·6	8·0	0·5	1·9	0·8	4·8
H-25, *H-26*	32	M	Lymphosarcoma	42·5	8·0	22·5	6·0	6·0	57·9	6·0	21·7	14·4	18·3
H-9	55	M	Bronchial carcinoma						10·7	2·9	3·7	0·6	4·3
H-74, *H-75*	68	M	Tuberculosis	8·7	2·7	4·6	1·8	1·9	26·1	5·6	10·2	5·5	4·8
H-67, *H-63*	66	M	Tuberculosis	11·1	3·1	4.4	1·8	1·8	12·3	4·5	2·8	2·7	2·3
H-62, *H-66*	65	M	Cardiac failure	5·2	0·9	2·7	0·7	0·9	6·5	1·8	2·7	1·1	0·9
H-81	67	M	Cardiac failure						13·1	5·5	2·4	2·9	2·4
H-84	65	M	Cardiac failure						5·4	1·1	0·8	0·8	2·7

dehydrogenase activity was determined in the starting material as well as the individual fractions. Enzyme concentration was expressed as μmoles diphosphopyridine nucleotide oxidized per hr. per ml. Recoveries, that is, the sum of the concentrations of the individual fractions, were within 70 and 110 per cent of the starting material. To eliminate differences in recoveries, all results were adjusted to total serum lactic dehydrogenase activity (Table 1).

The results may be summarized as follows:

(1) Higher lactic dehydrogenase concentration in effusions as compared to the corresponding sera is frequently encountered in cancer and local infections, whereas in congestion, enzyme concentration is equal or lower in the transudate[1]. Due to overlap, little diagnostic significance can be attributed to these differences in total enzyme activity.

(2) In non-infected non-malignant effusions, heterogeneity of lactic dehydrogenase mirrors the serum pattern as shown in Fig. 1*a*.

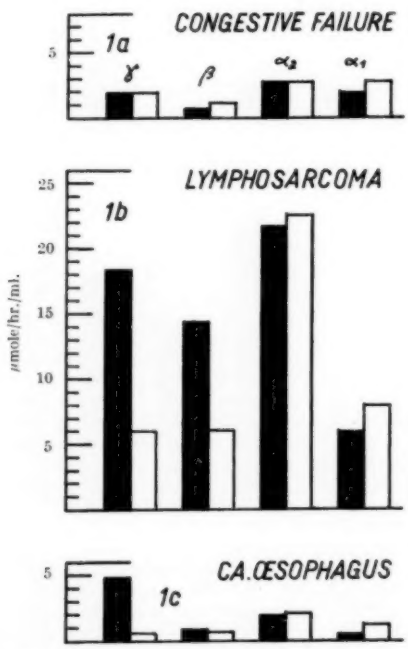


Fig. 1. Heterogeneity of lactic dehydrogenase. Black columns, effusions; white columns, serum. (*a*) 58-year-old male with congestive heart failure (serum lactic dehydrogenase 5·2 units; effusion lactic dehydrogenase 6·5 units). (*b*) 31-year-old male with generalized lymphosarcoma (serum lactic dehydrogenase 43 units; effusion lactic dehydrogenase 58 units). (*c*) 71-year-old male with metastasizing œsophageal carcinoma (serum lactic dehydrogenase 4·0 units; effusion lactic dehydrogenase 8·0 units)

(3) In malignant effusions, γ-lactic dehydrogenase is markedly increased in absolute value as compared with benign effusions, as well as relatively in comparison to the corresponding serum. The α_1 and α_2 enzymes reflect the serum pattern. The increase in the γ-fraction is independent of total lactic dehydrogenase, which may be very high, as in Fig. 1*b* (lymphosarcoma) or 'normal' (œsophageal carcinoma) as in Fig. 1*c*.

(4) If hæmolysis occurs, the heterogeneity of the pattern is distorted in comparison to serum too, but the increase is restricted to the α_1 fraction. In hæmolysed erythrocytes only an α_1 and α_2 enzyme can be demonstrated[3,6].

These findings indicate that in effusions the presence of an increased amount of lactic dehydrogenase migrating as a γ-globulin is suggestive of cancer. The number of samples investigated is too small to justify a definite statement, but the importance of the demonstration of a quantitative and possibly qualitative difference between a normal and a malignant body fluid is obvious. Warburg and Christian[9] first demonstrated abnormal leakage of glycolytic enzymes from cancer cells. Our observations suggest that, independent of the type of cancer, the leaking enzyme is primarily a γ-lactic dehydrogenase, which may be due either to selective leakage or to a distortion of the cellular lactic dehydrogenase pattern in cancer. Wieland and Pfleiderer[2] reported an increase in the slow-migrating lactic dehydrogenases ('γ-enzyme') in one bronchial carcinoma as compared with normal lung tissue, but further investigations will be necessary to settle this question. The discrepancy between effusion and serum pattern in malignancy is puzzling too. The lack of an increase in γ-lactic dehydrogenase in serum may be due to dilution, to local destruction by peptidases or to denaturation.

This work was supported by the Schweizerischer Nationalfond zur Förderung der Wissenschaftlichen Forschung (No. 1968).

R. Richterich
K. Zuppinger
E. Rossi

Medizinisch-chemisches Institut und Kinderklinik, Universität, Berne.

[1] Wroblewski, F., and Wroblewski, R., *Ann. Int. Med.*, **43**, 813 (1958).
[2] Wieland, T., and Pfleiderer, G., *Biochem. Z.*, **329**, 112 (1957).
[3] Vesell, E. S., and Bearn, A. G., *Proc. Soc. Exp. Biol. Med.*, **94**, 96 (1957).
[4] Richterich, R., Gautier, E., Zuppinger, K., Egli, H., and Rossi, E., *Klin. Wschr.*, **39**, 346 (1961).
[5] Wieme, R. J., *Clin. Chim. Acta.*, **4**, 46 (1959).
[6] Hess, B., and Walter, S. I., *Klin. Wschr.*, **38**, 1080 (1960).
[7] Kunkel, H. G., *Methods of Biochem. Anal.*, **1**, 141 (1954).
[8] Kubowitz, F., and Ott, P., *Biochem. Z.*, **314**, 94 (1943).
[9] Warburg, O., and Christian, W., *Biochem. Z.*, **314**, 399 (1943).

An Antigen in Lesions of Molluscum Contagiosum

OUCHTERLONY's technique of agar diffusion[1] has made it possible to demonstrate the presence of certain viral antigens and antibodies not identifiable by other serological means. Datt and Orleans[2], in 1958, found an antigenic substance common to vaccinia and pig pox; Taylor-Robinson and Rundle[3] have demonstrated a common antigen in chicken pox and herpes zoster. These successes prompted a re-investigation of the antigenicity of molluscum contagiosum, a large virus that produces benign skin tumours in man.

The method of Taylor-Robinson and Rundle[3] was used. Skin curettings of molluscum lesions were pooled, ground and lyophilized to give a crude antigen. Four different antigen samples were prepared; 50–150 lesions from two to five patients comprised each sample. The donors were used only once; no anæsthesia was required. The antigen suspended in saline, 3–4 mgm./ml. or 30–40 mgm./ml., was put in the central reservoir of agar plates prepared for immunological study (1 per cent (w/v) Difco agar dissolved in isotonic saline and phosphate buffer *p*H 7·3 and containing merthiolate 1: 10,000; wells 11 mm. apart and 5 mm. deep). Sera from (1) patients infected with molluscum contagiosum; (2) adults without the disease, and (3) uninfected children were put in surrounding wells. The plates were examined daily for 2–3 weeks.

A single precipitation band (Fig. 1) appeared in 9 of 21 patients (43 per cent), 8 of 73 adults (11 per cent), and 0 of 11 children, and was usually evident within 48 hr. Weak concentrations of antigen (4 mgm./ml.) gave positive reactions only with sera from patients having numerous (more than 50) lesions of molluscum contagiosum. All antigens displayed equal activity. Comparable concentrations of lyophilized normal skin curetted from the same donors or from normal subjects gave consistently negative results. Ground, lyophilized human warts showed no antigenicity in this system. Of the positive reactors in the adult group, four had markedly raised serum globulin-levels related to multiple myeloma or internal cancers. A fifth subject with a high serum γ-globulin had a history of infectious hepatitis. One additional subject had a false-positive serological test for syphilis without a significant electrophoretic abnormality. These alterations were absent in sera from patients with molluscum contagiosum.

These findings indicate the presence of an antigen capable of inducing antibody formation in the lesions of molluscum contagiosum. This may conform to the heat-labile antigen–antibody system reported by Mitchell[4]; he noted weak complement fixation reactions of crude molluscum antigen with sera from 3 of 14 patients infected with the virus. About half our patients with the disease had demonstrable antibodies; they were more prevalent in those patients with extensive lesions. While the positive reactions in adults can be explained on the basis of prior unrecognized infection[5], it seems curious that 50 per cent of the positive reactors had high serum globulin associated with internal malignancy; the possibility exists that a cross-reacting tumour antibody gave the observed precipitation bands. The non-infected children had been confined to an isolated ward and presumably had never been exposed to molluscum contagiosum.

Work is in progress to determine whether the crude antigen is a purely viral substance or an altered tissue antigen.

This work was supported in part by a grant from the Committee on Research, University of California School of Medicine.

WILLIAM L. EPSTEIN
IRENE P. SENECAL
ALAN M. MASSING

Division of Dermatology, Department of Medicine, University of California School of Medicine, San Francisco.

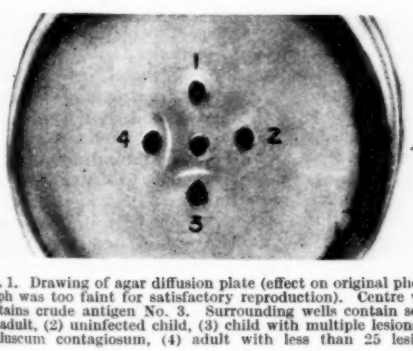


Fig. 1. Drawing of agar diffusion plate (effect on original photograph was too faint for satisfactory reproduction). Centre well contains crude antigen No. 3. Surrounding wells contain sera: (1) adult, (2) uninfected child, (3) child with multiple lesions of molluscum contagiosum, (4) adult with less than 25 lesions

[1] Ouchterlony, O., *Acta Path. Microbiol. Scand.*, **25**, 186 (1948).
[2] Datt, N. S., and Orleans, E. S., *Immunol.*, **1**, 81 (1958).
[3] Taylor-Robinson, D., and Rundle, C. J. M., *Brit. J. Exp. Path.*, **40** (5), 517 (1959). Crumpton, M. J., and Davies, D. A. L., *Proc. Roy. Soc.*, B, **145**, 109 (1956).
[4] Mitchell, J. C., *Brit. J. Exp. Path.*, **34**, 44 (1953).
[5] Blank, H., and Rake, G., *Viral and Rickettsial Diseases of the Skin, Eye, and Mucous Membranes of Man* (Boston: Little, Brown and Co., 1955).

Leukæmia in the Progeny of Mice inoculated with a Leukæmogenic Virus

TEN years ago Gross first reported a raised incidence of leukæmia in mice of a low-leukæmic strain (*C3H*) inoculated when newly born with a cell-free extract of leukæmic tissue from a high-leukæmic strain (*AK*)[1]. He also observed a raised incidence in the progeny of the inoculated mice[2]. Since then, the first observation has been several times confirmed, but the second has not been reported from any other laboratory.

In the course of recent work in this Department on another leukæmogenic agent, the virus which Moloney isolated from the sarcoma 37 [3], several groups of stock albino mice ('Schofields') were injected when newly born with cell-free filtrates prepared from tissues of leukæmic mice of various breeds which had received a tissue culture-passaged sample of Moloney's virus[4]. In one experiment a new-born litter of 7 stock albinos (2♂, 5♀) were injected with such a filtrate. Leukæmia began to appear at 12 weeks of age, and 6 mice had developed leukæmia by 20 weeks. Before the appearance of the first leukæmia the mice were brother-sister mated; of their progeny of 18 (9♂ and 9♀) born when the parents were approximately 9 weeks old, 11 have become leukæmic between the 11th and 20th week of age. In the second experiment 7 new-born litters of stock albinos (a total of 46 mice) were inoculated with another filtrate. Leukæmia began to appear at the 11th week, and the incidence reached 50 per cent at 21 weeks. Among the mixed progeny (total 27) of these 7 litters, also born when their parents were about 9 weeks old, 9 leukæmias have appeared between the 11th and 20th week of age.

In a long experience of this breed of mice we have never seen spontaneous leukæmia as early as this. It occurs occasionally between 6 and 10 months of age: the cumulative incidence for the first 12 months is about 3 per cent.

Three contemporary control groups of stock albino mice of the same breed may be directly compared with the inoculated groups and their progeny:

(1) 32 untreated controls, 22 weeks old. No leukæmias.

(2) 46 mice inoculated with the same Moloney filtrate heated for ½ hr. at 80° C. These are now 24 weeks old. One leukæmia appeared at 22 weeks.

(3) 12 progeny of (2), now 16 weeks old. No leukæmias.

It would be premature to speculate at present about the mechanism of this phenomenon. Various possible modes of transmission are being investigated.

M. H. Salaman
J. J. Harvey

Cancer Research Department,
London Hospital Medical College,
Whitechapel,
London, E.1.

[1] Gross, L., *Proc. Soc. Exp. Biol. Med.*, **76**, 27 (1951).
[2] Gross, L., *Proc. Soc. Exp. Biol. Med.*, **78**, 342 (1951).
[3] Moloney, J. B., *J. Nat. Cancer Inst.*, **24**, 933 (1960).
[4] Salaman, M. H., Rowson, K. E. K., and Harvey, J. J. (in the press).

IMMUNOLOGY

Antibodies to Trophoblasts during the Post-Partum Period

Recent investigation of homograft tolerance has stimulated speculation about the immunological relationship between mother and fœtus during pregnancy[1,2]. Since the fœtus with its placenta is genetically different from the maternal host, it can be looked upon as a homograft tolerated by the mother for nine months. Fundamental to the support of such a concept is the demonstration of an immune response by the mother to her products of conception at some time during or after pregnancy. In human beings, the syncytiotrophoblasts are the fœtal cells in direct contact with the maternal bloodstream. They invade the bloodstream and disappear[1]. It would seem, therefore, that an immune response by the mother would most likely be directed towards trophoblasts. Although experimental homograft immunity is mediated primarily through a cellular response by the host[3], the direct contact between trophoblasts and maternal blood might stimulate a humoral response as well. The large mass of trophoblastic tissue in the placenta could absorb circulating antibody effectively. This might explain previous reports of failure to detect antibodies to placenta during pregnancy[4,5]. After delivery of the fœtus and placenta, the absence of this absorptive surface might allow any anti-trophoblastic antibody to remain in the bloodstream, where it could be detected by appropriate methods.

Based on these considerations, attempts were made to demonstrate anti-placental antibody in the sera of post-partum patients. Portions of placentas obtained from normal deliveries were immediately washed in EDTA-buffered saline (0·01 *M* EDTA adjusted to *p*H 7·2) until largely free of blood. A water-soluble extract of the homogenized tissue was prepared and lyophilized for storage. Smaller portions of the fresh placenta were quick-frozen in a dry-ice-ethanol bath and stored in a dry-ice chest. The patient's blood was drawn on the fourth post-partum day and the serum tested against her own placenta in every case.

Attempts to detect a precipitating antigen-antibody system with various concentrations of lyophilized placental extracts and post-partum sera were unsuccessful. In these experiments, the direct precipitin, Ouchterlony agar diffusion and agar immunoelectrophoresis techniques were employed. Concentrated globulin fractions of the sera also failed to give a precipitin reaction. Inconclusive hæmagglutination patterns have been obtained so far, using tanned sheep red blood cells sensitized[6] with various dilutions of placental extract and tested with doubling dilutions of post-partum serum.

Positive results were obtained, however, using the fluorescent antibody method of Coons on tissue sections[7]. The globulin fraction of post-partum serum was obtained by precipitation with 20·4 per cent sodium sulphate, tagged with fluorescein *iso*thiocyanate, and absorbed with mouse and rat liver powders. Globulin from the serum of a non-pregnant normal woman was treated similarly as a control. The frozen placental samples were sectioned at 4μ in a cryostat, dehydrated in alcohol for 30 sec., and stained at room temperature. Treatment of the sections with tagged post-partum globulin for 30 min. resulted in the localization of fluorescence in all cellular elements of the villi as viewed by ultra-violet microscopy. When the section was first flooded with untagged post-partum globulin for 30 min., sites of fixation on the tissue were blocked and subsequent treatment with the tagged material failed to result in fluorescence.

Several factors unrelated to an immune response by the mother to the placenta could play a part in the fixation of globulin by placental tissue. Globulin was fixed non-specifically despite prior absorption with rat and mouse liver powders, as illustrated by the presence of some fluorescence after staining with tagged globulin from a normal non-pregnant woman. Another factor was the known ability of the placenta to transport globulin from the maternal blood to the fœtal circulation[8]. The active sites of such a transport mechanism in the section might bind tagged globulin independently of any antigen-antibody system. A third factor was the possible presence of blood-group substances in the placental tissues. These could also play a part in the fixation of post-partum serum.

These possible explanations were eliminated by flooding the sections with untagged globulin from a non-pregnant normal woman of blood group *O*. Sites of non-specific binding of protein would thus be blocked. Sites involved in the mechanism of globulin transport would also be blocked. Any *A* or *B* blood-group substance on the placental section would combine with the anti-*A* and anti-*B* antibody present in the type *O* globulin and would be unavailable for further reaction. When such a pre-treated section was treated with tagged globulin from a non-pregnant woman, no fluorescence was seen, but treatment with tagged globulin from a post-partum woman resulted in fluorescence which was limited to the cytoplasm of the syncytiotrophoblasts (Fig. 1). This reaction has been observed in each of five patients of differing parities and blood-groups thus far studied.

The evidence favours the interpretation of the binding of tagged globulin from post-partum sera by

Fig. 1. Frozen section of human placenta treated with globulin from serum of a non-pregnant woman of blood group *O*, then stained with fluorescein-tagged globulin from the serum of the woman who delivered the placenta. Under ultra-violet excitation, only syncytiotrophoblastic cytoplasm fluoresces. (× 90)

the syncytiotrophoblastic cytoplasm as an immune phenomenon. An investigation designed to delineate the time of origin of this reaction with respect to possibly related events such as labour, pre-eclampsia and abortion is under way.

This work was partially supported by a grant from the Population Council, Inc.

J. F. Hulka*
K. C. Hsu
S. M. Beiser

Department of Microbiology and the Department of Obstetrics and Gynæcology, Columbia University College of Physicians and Surgeons, New York, 32.

* Josiah Macy, Foundation Fellow.

[1] Douglas, G. W., Thomas, L., Carr, M., Cullen, N. M., and Morris, E., *Amer. J. Obstet. and Gynec.*, **78**, 960 (1959).
[2] Bardawil, W. A., and Toy, B. L., *Ann. N.Y. Acad. Sci.*, **80**, 197 (1959).
[3] Hildeman, W. H., and Medawar, P. B., *Immunology*, **2**, 44 (1959).
[4] Thornes, D. R., thesis, University of Dublin (1958).
[5] Olivelli, F., and Ruggieri, P., *Minerva Gin Tor*, **10**, 953 (1958).
[6] Stavitsky, A. B., *J. Immunol.*, **72**, 360, 368 (1954).
[7] Coons, A. H., and Kaplan, M. H., *J. Exp. Med.*, **91**, 1 (1950).
[8] Edsall, G., *Ann. N.Y. Acad. Sci.*, **66**, 32 (1956).

Specific Inhibition of Anti-D Antibody by Colominic Acid

In ref. 1 it is reported that certain nucleotides specifically inhibited anti-*Rh* antibodies, and in ref. 2 that there is specific inhibition by certain monosaccharides and substances such as rutinose and streptomycin. Although some differences in the effect of these substances on anti-*C*, anti-*D* and anti-*E* were observed by both groups of workers, in general a substance that inhibited one anti-*Rh* serum also inhibited the others. More recently, Dodd, Bigley and Geyer[3] have reported that *N*-acetyl neuraminic acid and certain related compounds inhibit anti-*D* but not anti-*C* or anti-*E*; this is a greater degree of specificity than that claimed in the first two reports.

We have confirmed the work of Dodd, Bigley and Geyer. In addition to the compounds tested by them, we have found that colominic acid, a substance produced by certain strains of *Escherichia coli* and shown probably to be a polymer of *N*-acetyl neuraminic acid[4,5], inhibits anti-*D*, but not anti-*C* or anti-*E*, in relatively low concentration (0·006 *M*).

In addition to the interesting implications of these observations for the structure of the *Rh* antigens, we believe our last observation is of possible practical importance. Colominic acid is stated[5] to be non-toxic and non-antigenic, and could undoubtedly be produced in quantity if necessary. We are therefore proposing to try it clinically to neutralize the anti-*D* in *Rh*-sensitized pregnant women and thus possibly prevent erythroblastosis in the unborn child.

This work was supported by research grants (*H*–1076 (*C*8), *RG*–4704 (*C*4) and *RG*–6939) from the National Institutes of Health, and a research grant (*NSF* G–5131) from the National Science Foundation. We are indebted to Dr. W. F. Goebel for a sample of colominic acid and a culture of *E. coli* K_{235}.

William C. Boyd
Egli Reeves

Department of Immunochemistry, Boston University School of Medicine, Boston 18, Massachusetts.

[1] Hackel, E., Smolker, R. E., and Fenske, S. A., *Vox Sang.*, **3**, 402 (1958).
[2] Boyd, W. C., McMaster, M. H., and Waszczenko-Zacharczenko, E., *Nature*, **184**, 989 (1959).
[3] Dodd, M. C., Bigley, N. J., and Geyer, V. B., *Science*, **132**, 1398 (1960).
[4] Barry, G. T., and Goebel, W. F., *Nature*, **179**, 206 (1957).
[5] Barry, G. T., *J. Exp. Med.*, **107**, 507 (1958).

BIOLOGY

Hydrogen Ion Concentration, Plankton and Fish in Fresh-water Eutrophic Lakes of India

Das *et al.*[1] have recently published results on the quantitative and qualitative aspects of plankton of some eutrophic fresh-water lakes of Uttar Pradesh, India; but no satisfactory information is available regarding the physico-chemical factors bearing upon them. Some work on related fields has been done by Chacko *et al.*[2] and Alikunhi[3], but their results deal only with part of the season in fish-hatching tanks and small bodies of water. In order to get detailed information regarding *p*H and plankton, regular weekly quantitative hauls were made and water samples obtained in 'Pyrex' glass-stoppered bottles. Hydrogen ion concentration was determined for each sample colorimetrically by using Helige comparators and Helige indicator solutions in daylight so far as possible in the field. Carbon dioxide was estimated by making use of phenolphthalein as indicator and the titration was done in Nessler jars in the usual way.

The investigations show that *p*H values range from 7·2 to 9·2 during the year. A high *p*H is observed in the months of July and August, when the average is about 8·6. But the values decrease during September and October and reach 7·4 in the last two weeks of October. November and December maintain rather constant values, but January shows a sudden increase in *p*H, reaching 8·8 in the third week; finally, a further rise occurs and reaches a maximum of 9·2 in the first week of February. In March *p*H values are still as high as 9·0, but show a fall to 8·0 in the last week of April. Finally, in the months of May and June, there is sharp decline in *p*H to 7·2.

The plankton records for the same period show that high *p*H values in the months of July and August coincide with the first monsoon phytoplankton peak. Then the decrease in *p*H during September–December can be attributed mainly to the zooplankton peak, along with the decomposition of

organic matter caused by death and decay of zooplankton. Again a high *p*H in the months of February and March was observed to be accompanied by the blooming of Myxophyceae, causing an increase in phytoplankton. The lowest *p*H values were obtained during May and June, when there was an abundance of zooplankton and lack of phytoplankton.

It has also been observed that high *p*H values of water coincide with low values for carbon dioxide, and with the phytoplankton peaks, while low *p*H values coincide with high concentration of carbon dioxide in water during the zooplankton peak periods. This appears to be due to the fact that increase in phytoplankton causes photosynthetic activity which uses up dissolved carbon dioxide, and thus the *p*H values are raised in February and March. In the summer months, due to the concentration of aquatic animals (fishes, molluscs, etc.) in the decreased amount of water, combined with the abundance of zooplankton, the concentration of carbon dioxide increases and *p*H values reach a minimum. It is thus clear that, generally, high *p*H means high phytoplankton and low zooplankton; whereas low *p*H means low phytoplankton and high zooplankton in ordinary circumstances.

It has recently been observed (Das, unpublished work) that more fishes are present in natural lakes with a *p*H range of 7·2–8·2, but decline rapidly in highly alkaline waters which remain above *p*H 9. As no Indian eutrophic lakes have been found with a *p*H less than 7·2, fish fertility appears to be indirectly dependent on the *p*H of such bodies of water. Thus a change of environment due to change of *p*H may affect the productivity of fish in fresh-water lakes and tanks. This aspect is now being studied.

S. M. Das

Department of Zoology,
University of Lucknow.

[1] Das, S. M., *et al.*, *Proc. Nat. Acad. Sci.* (India), **26** (1); **26** (4); **27** (3); **29** (4); **29** (4) (1956–59).
[2] Chacko, P. I., *et al.*, *Symp. Marine and Freshwater Plankton in the Indo-Pacific, Bangkok* (Unesco, 1954).
[3] Alikunhi, K. H., *et al.*, *Ind. J. Fisheries*, **2** (1955).

Littoral Interstitial Species from Anglesey representing Three Families new to Britain

In 1956 a brief investigation by Dr. B. Swedmark of beaches in the Anglesey area revealed a well-developed microfauna. A more intensive survey is now in progress, and many typical interstitial species have been found.

Some of the most interesting recent discoveries have been made in the low-water neap zone at Traeth Bychan, a gently sloping beach of coarse sand on the east coast of Anglesey. Three small molluscs belonging to the Pseudovermidae, a nudibranch family new to Britain, have been found in this habitat. They resemble *Pseudovermis schulzi* Marcus and Marcus more closely than any other species described. All three measured 3–3·5 mm. in length when extended, but were unfortunately immature. The shape of the radular plates together with the presence of eyes seem to confirm the identification as *P. schulzi*, but there were as many as fifteen body papillæ and an equal number of cnidosacs (Fig. 1). A maximum of five papillæ was given as a diagnostic feature in the original description. The cnidoblasts were all of one type, penetrants 5·5μ long and 4μ broad with a thread up to 70μ long when discharged.

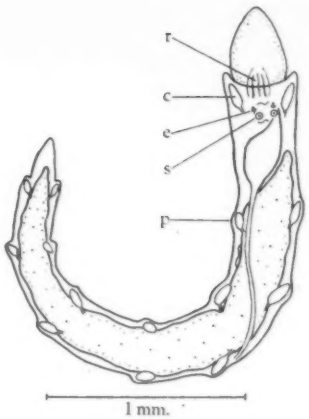


Fig. 1. *Pseudovermis* sp. from Anglesey. Dorsal view. *c*, cnidosac; *e*, eye; *p*, body papilla; *r*, radula; *s*, statocyst

They were found to be identical in size and structure with those of the hydrozoan *Halammohydra vermiformis* Swedmark and Teissier, which has been found in the same area of beach. It is thus highly probable that this *Pseudovermis* species preys upon *H. vermiformis*. Apart from the suggestion by Dr. E. Marcus that the Brazilian *Pseudovermis salamandrops* Marcus derives its nematocysts from *Psammohydra nanna*, Schulz[1], no previous information concerning the food of pseudovermids is available.

A second species of *Halammohydra*, *H. schulzi* Remane, has been found in the Menai Straits among coarse shell gravel exposed at low water on spring tides. The family Halammohydridae had not previously been recorded from Britain, but an unidentified species has since been reported from the Isles of Scilly[2].

Last August a sample of sand from Traeth Bychan which contained several young *H. vermiformis* yielded a curious interstitial annelid. Further examples of this animal recovered recently prove it to be the sedentary polychæte *Psammodrilus balanoglossoides* Swedmark belonging to the Psammodrilidae Swedmark. This constitutes the third new family from this one habitat to be added to the British fauna list.

P. J. S. Boaden

Marine Biology Station,
Menai Bridge,
Anglesey.

[1] Marcus, E. du B.-R., *Bol. Fac. Filos. Cienc., Zool.*, **18** (1953).
[2] Harvey, L. A., and Wells, J. B. J., *Nature*, **189**, 944 (1961).

Response to Series of Subliminal Stimuli; an Ethological Method for investigating the Properties of Nervous Systems

Queen ants of genus *Formica* react to mechanical disturbance of the nest by running downwards to the deepest chambers. In laboratory colonies a series of shocks could result in a reaction, although one similar shock was too small to produce any visible effect.

To investigate this further, three separate queens of *F. fusca* L., without workers, were subjected at the same time to series of nearby taps on the bench with constant periodicity throughout each series and constant strength throughout this work. The periodicity was changed for different series and the

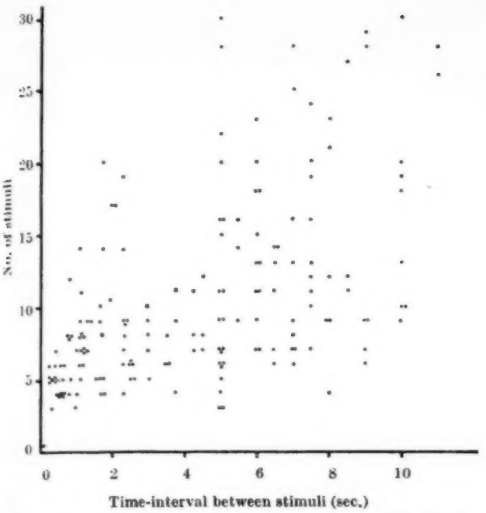


Fig. 1. The relation between the number of mechanical shocks necessary to produce an escape reaction by queen *Formica fusca* L., and the time-interval between the shocks

range of time-interval (t) between taps was 0·2–11·0 sec. Fig. 1 shows the number of taps (n) needed for a reaction plotted against t (regression analysis of log n on t; $P < 0{\cdot}001$).

There are several causes of the wide variance of these results, including: (i) differences in the internal state of the animals; (ii) differences in their stance; (iii) changes in temperature (range 21·3–24·5° C., mean 23·0° C.); (iv) other mechanical shocks in the same building cannot be completely discounted, but were small compared with the deliberate stimulus, and the reaction always occurred immediately after a deliberate stimulus. These causes are not expected to bias the results and are, for the present, ignored, although the method may provide opportunities for measuring the effect of important factors.

Various difficulties in observation and interpretation were encountered. The reaction varied from a change of posture (a raising of the head and thorax with extension of the antennæ) to rapid running, and in one in eight experiments a queen did not respond in spite of prolonged stimulation. This queen was ignored in this particular experiment, if it had not responded to three times as many taps as taken for the last of the others to react. Only one response, out of 172, was given to the first stimulus and the other queens in this experiment took 6 and 7 taps to react. Habituation did not appear to be an important factor, but at least 30 min. was allowed to elapse between experiments. Isolation of the queens from workers and brood was not relevant either, since a colony exposed to the same conditions at the same time showed similar behaviour with the queen usually reacting before the workers.

Evidently, there is a threshold of some nervous change which has to be reached before reaction occurs. This change shows temporal summation and can be retained for more than 10 sec. although it is gradually lost after stimulation. Exponential decay fits the results better than linear decay and, if it is exponential, its average half-life is estimated to be at least 30 sec. (since, if when $t = 0$, $n = N$, there is a vertical asymptote at the value of t during which $1/N$th of the change decays).

Unless facilitation of sense organs to subliminal stimuli can contribute significantly, these long periods of activation are due to summation and decay of activity in a nervous centre controlling escape behaviour. This centre may be an 'instinctive centre' in the sense of Tinbergen[1] and the activity may be related to the 'stored energy' of Lorenz[2] which is also required to reach a threshold before setting off behaviour.

A. J. Pontin

Bedford College,
Regent's Park, N.W.1.

[1] Tinbergen, N., *Symp. Soc. Exp. Biol.*, Cambridge, **4**, 305 (1950).
[2] Lorenz, K. Z., *Symp. Soc. Exp. Biol.*, Cambridge, **4**, 221 (1950).

Enhanced Plant-Growth with Two Systemic Insecticides

In the search for better methods of control of the red-legged earth-mite, *Halotydeus destructor* (Tuck.) (Acarina : Eupodidae), and the lucerne flea, *Sminthurus viridis* (L.) (Collembola : Sminthuridae), seeds of Dwalganup subterranean clover, *Trifolium subterraneum* L., were treated with the systemic insecticides demeton and methyl demeton before sowing. The seed, dampened with milk, was tumbled in a container with 8 per cent of its weight of a charcoal-based dust containing 50 per cent of the insecticide. Seeds were sown, at the rate of 50 per 8-in. earthenware pot, in sand fertilized with potato 'E' manure at the rate of 1 cwt./acre. (Potato 'E' manure contains 3·5 per cent N, 15·5 per cent P_2O_5 and 8 per cent K_2O.) The pots, of which there were three for each treatment, were kept in a glasshouse and watered at least every second day.

The emergence was 96 per cent in untreated seed; 93 per cent in the demeton treatment and 59 per cent in the methyl demeton treatment. Seedlings removed from the demeton treatment were toxic to both pests for up to 11 weeks after sowing, whereas toxicity was lost in the methyl demeton treatment after about 7 weeks. Demeton-treated seeds produced pale cotyledons and stems, whereas those treated with methyl demeton produced plants which were more heavily pigmented than normal. In both insecticide treatments the early growth of the seedlings was slightly retarded. Despite this, the plants arising from the treated seed later grew more vigorously than those in the control pots.

Three months after sowing, all plants were carefully removed from the pots and the weights of the green tops and of the roots recorded. The results are shown in Table 1. The increased yield in the pots sown with seed treated with demeton and methyl demeton amounted to an additional 39 per cent and 24 per cent respectively ($P < 0{\cdot}05$). There was a highly significant increase in the weights of the individual plants ($P < 0{\cdot}001$), but no significant differences in weight of roots. In the methyl demeton treatment it is possible that part at least of the increase in individual plant weight may have been a response to reduced seedling competition.

There was virtually no insect or mite attack on the plants in the glasshouse, and certainly insufficient to account for such large differences of yield. Small increases in growth have since been observed in outdoor pot tests with phorate ('Thimet') and *PP*175 ('Sayfos'), although no precise measurements were

Table 1. Effect of Demeton and Methyl Demeton applied as a Seed Treatment on the Yield in Pots of the Green Tops and Roots of Subterranean Clover

Treatment	No. plants at harvest	Yields (gm.)			
		Total tops	Mean tops per plant	Total roots	Mean roots per plant
Control	127	180·8*	1·42†	137·1	1·09
Demeton	125	250·4*	2·04†	148·0	1·18
Methyl demeton	86	223·7*	2·60†	126·1	1·47

* $P < 0.05$ † $P < 0.001$

taken. The phorate used was a 44 per cent charcoal-based dust whereas the *PP*175 was an 80 per cent wettable powder. Similar unexplained increases in growth following seed treatment with phorate and demeton were reported[1,2].

Clearly, these insecticide seed-treatments had some marked beneficial effect other than the direct killing of insect or mite pests. Perhaps there was some influence on the rhizosphere microfauna or flora, or possibly the transmission of virus diseases was inhibited. Whatever the explanation, there appears to be some real response worthy of further investigation.

M. M. H. Wallace

Commonwealth Scientific and Industrial Research Organization, Division of Entomology, Western Australian Regional Laboratory, Nedlands, Western Australia.

[1] Guyer, G. E., Brown, H. M., and Wells, A., *Quart. Bull. Mich. Agric. Exp. Stat.*, **40** (1958).
[2] Brown, H. E., *FAO Plant Protection Bull.*, **5** (1957).

Effects of Gibberellic Acid and Allogibberic Acid on Flowering and Internode-Length in *Pisum sativum*

Several workers[1,2] have shown that gibberellic acid causes an increase in internode-length in peas, especially in the dwarf (*le*) varieties where the increase may be as great as 500 per cent. In addition we have shown that gibberellic acid delays flowering by 1–2 nodes in tall (*Le*) or dwarf (*le*) and late (*Sn*) or early (*sn*) varieties[3]. Segregation of alleles at the *Le* and *Sn* loci has a number of overlapping effects[3]. It thus appears that genes at both loci may be working by way of some aspect of gibberellin metabolism. Accordingly we have tested the effect on flowering and stem-length of peas of a number of compounds related in structure to gibberellic acid. We are indebted to Dr. P. W. Brian (Imperial Chemical Industries, Ltd.) for the gift of the compounds used.

The compounds were applied as a solution in absolute ethanol to the dry seed of the early, dwarf variety, Massey, at a concentration of 0·4 μgm. per μl. Doses of 1, 2, 8, 16 and 32 μgm. were regulated by varying the volume of the solution applied by means of a micropipette. The ethanol rapidly evaporates leaving the compound on the testa of the seed. The seeds were germinated in moist vermiculite and transplanted as seedlings into soil. Comparable doses of pure ethanol had no effect on the subsequent growth of the plant.

Compounds tested include methyl gibberellate, gibberellin *C*, gibberellenic acid, gibberic acid and allogibberic acid. Methyl gibberellate produces similar responses on both flowering and stem-length to gibberellic acid, but is not so active. Gibberellin *C* and gibberellenic acid were inactive at doses of 1 and 2 μgm. per plant. At 32 μgm. they have an effect equivalent to a dose of less than 1 μgm. of gibberellic acid. The effects of these two substances are most probably attributable to contamination with gibberellic acid (Dr. Brian has informed us that it is very difficult to prepare pure samples of these compounds). Gibberic acid had no effect on either growth or flowering at any of the concentrations tested. Allogibberic acid had no effect on stem-length but appeared to cause a small delay (0·5 node) in flowering.

In a more detailed study of the effect of allogibberic acid, four chemical treatments (0, 1, 10 and 100 μgm. of allogibberic acid per plant) were combined factorially with two photoperiods (short days of 8 hr. and long days of 18 hr.). A randomized block design of 20 plants per treatment was used. Experimental procedure was the same as in the previous experiment except that the different doses were applied in each case in the same volume (10 μl.) of ethanol.

Flowering is delayed to a higher node by allogibberic acid. The delay shows a steady increase from zero at a dose of 1 μgm. to a maximum of 0·65 node (statistically significant at the 1 per cent level) at the highest dose of 100 μgm. per plant. In contrast, allogibberic acid does not alter internode-length at any of the concentrations tested. The photoperiod treatment and the photoperiod-chemical interaction are not significant for either node of first flower or internode length. The long- and short-day results have been combined and plotted in Figs. 1 and 2 together with results from a previous experiment on the effect of seed treatment with gibberellic acid on the same pea variety, Massey.

Fig. 1 illustrates that gibberellic acid and allogibberic acid both cause a delay in flowering. The effect of gibberellic acid is approaching a maximum

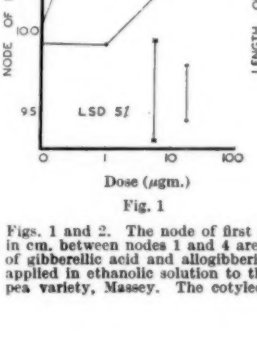


Fig. 1

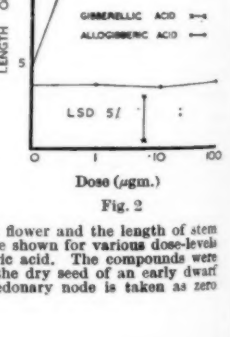


Fig. 2

Figs. 1 and 2. The node of first flower and the length of stem in cm. between nodes 1 and 4 are shown for various dose-levels of gibberellic acid and allogibberic acid. The compounds were applied in ethanolic solution to the dry seed of an early dwarf pea variety, Massey. The cotyledonary node is taken as zero

at a dose of 4 μgm. per plant, whereas the delay caused by allogibberic acid may still be increasing at the maximum dose of 100 μgm. The delay achieved with this highest dose is only about half the maximum delay achieved with gibberellic acid. Fig. 2 contrasts the effect of the two compounds on stem-growth. Two μgm. of gibberellic acid causes about a 400 per cent increase in stem-length whereas 100 μgm. of allogibberic acid has no effect. These results on stem-growth are in agreement with those of Cross[4] and Brian *et al.*[1,5].

The chemical structures of gibberellic acid, allogibberic acid and gibberic acid have been established by Cross *et al.*[6,7]. Chemically, allogibberic acid is a primary degradation product from gibberellic acid and differs from it only in ring *A*. Biologically, allogibberic acid has lost the power to stimulate elongation in dwarf peas but is still capable of delaying flowering. Gibberic acid, which is another degradation product derived from allogibberic acid by a Wagner–Meerwein rearrangement, seems to have lost both activities. The delay in flowering induced by gibberellic acid or allogibberic acid is not altered under different photoperiods and it is unlikely, therefore, that either substance is the flower inhibitor (colysanthin) which we have suggested is produced by the dominant *Sn* gene of late varieties of peas[3].

One of us (I. C. M.) acknowledges the assistance of a Commonwealth Scientific and Industrial Research Organization research studentship. The Rural Credits Division of the Reserve Bank of Australia has helped us by providing funds for technical assistance, apparatus and glasshouses.

I. C. Murfet
H. N. Barber

Botany Department,
University of Tasmania, Hobart.

[1] Brian, P. W., and Hemming, H. G., *Physiol. Plant.*, **8**, 669 (1955).
[2] Barber, H. N., Jackson, W. D., Murfet, I. C., and Sprent, J. I., *Nature*, **182**, 1321 (1958).
[3] Barber, H. N., *Heredity*, **13**, 33 (1959).
[4] Cross, B. E., *J. Chem. Soc.*, 4670 (1954).
[5] Brian, P. W., Grove, J. F., Hemming, H. G., Mulholland, T. P. C., and Radley, M., *Plant Physiol.*, **33**, 329 (1958).
[6] Cross, B. E., Grove, J. F., MacMillan, J., Mulholland, T. P. C., and Sheppard, N., *Proc. Chem. Soc.*, 221 (1958).
[7] Cross, B. E., Grove, J. F., MacMillan, J., and Mulholland, T. P. C., *Chem. and Indust.*, 954 (1956).

Susceptibility to Leaf Blight, caused by *Cochliobolus heterostrophus*, in Nigerian Varieties of Maize

Leaf blights on maize are prevalent, particularly in the forest areas of Southern Nigeria. One of the commonest of these pathogens is *Cochliobolus heterostrophus* (Dreschl.) Dreschl., already recorded in West Africa in 1928 in Ghana[1] and in 1933 in Nigeria[2]. The lesions develop in abundance on the leaves, from the lower ones upwards. A typically infected maize plant was taken at tasselling time and the area of the various leaves attacked by the fungus determined (Table 1).

The fungus causes a premature dying back of the leaves from the base onwards, around the time of tasselling. Heavily infected plants lose their lowest leaves earlier and have no more green leaf area within approximately 3 wk. from tasselling, that is, before the grains on the ears are properly formed.

Most of the varieties introduced as breeding material, mainly from the Americas, proved to be very susceptible to this fungus. The most susceptible variety was Coahuila-8 from Mexico. The lowest scores were obtained on the synthetic variety *ES2*, a local selection from the Mexican variety Rocamex 520–*C*. However, even this selection has a considerable reduction in leaf area, in seasons favourable to the pathogen.

Table 1. Leaf Area and Percentage of the Area attacked by *C. heterostrophus* of a Typically Infected Maize Plant. Leaves Numbered from Base to Top

Leaf No.	Leaf area (cm.²)	Percentage of leaf area attacked
1	487	20·1
2	619	11·4
3	818	9·8
4	902	10·9
5	938	8·0
6	993	10·4
7	1,037	8·8
8	988	4·2
9	905	5·6
10	825	2·6
11	758	3·1
12	741	0·6
13	561	0·3
14	431	1·1
15	239	0·8
Total	11,242	6·3

For the purpose of a survey, a collection was made of West African maize varieties[2] of which approximately 650 are maintained at Moor Plantation, Ibadan. The main part of this collection consists of Nigerian varieties. Those local varieties of maize were grown at Ibadan during the early season of 1959 (March–July) with 30 plants per variety. Each plant was assessed quantitatively on the occurrence of lesions caused by *C. heterostrophus* with the aid of an arbitrary key. Three leaves were assessed per plant and a mean calculated. Three levels of attack were recognized, roughly corresponding with 0–1 per cent (intensity 1), 2–5 per cent (intensity 2) and 6–10 per cent or more (intensity 3) of the leaf area attacked by the pathogen. This was done in order to obtain information on possible sources for resistance.

The early season of 1959 was favourable for the development of the fungus and provided an excellent opportunity to screen the collection on resistance. The results have been mapped by indicating the average intensity score of *C. heterostrophus* on the various maizes on the place of their origin (Fig. 1).

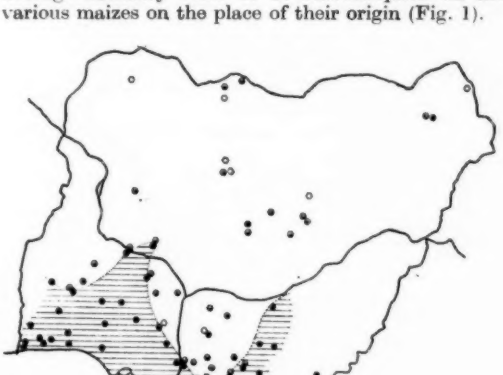


Fig. 1. Map of Nigeria showing the distribution of susceptibility and resistance to *Cochliobolus heterostrophus* in local maize varieties. ●, Intensity 1; ◒, intensity 2; ○, intensity 3 (scores are mapped on the place of origin of the varieties)

It is noteworthy that, apart from a few exceptions, the more resistant varieties (intensity score 1) have their origin in the forest belt of the south (hatched area on Fig. 1). This area comprises the main maize-producing area of Nigeria in the drier parts of the rain forest and derived savannah of the western region[4]. East of the River Niger, however, a broad front of less-resistant varieties invades the densely populated areas of the eastern region and reaches very far south. Within this triangle the varieties have white or yellow, floury (soft endosperm) or flinty (hard endosperm) grains. The majority of the western region varieties, however, are white and soft-grained. The same is true for varieties in the south and south-east of the eastern region. The northern maize varieties, however, are typically yellow and hard-grained.

Trade and accidental movements of maize grains could be responsible for the phenomenon of maize varieties susceptible to leaf blight in a zone where natural selection would have eliminated those and actually did to the west, south and south-east of the area. In several places just north of this wedge (Keffi, Lafia) 'Southern' floury varieties are cultivated for sale on markets where 'Southerners' have caused a demand for those grains, although the local population often dislikes this type. It is also known that a trade of maize occurs within the area from north to south[5], from the less to the more densely populated areas (for example, from Idoma and Igala divisions towards Onitsha).

It is suggested that the resistance to the Nigerian strains of *C. heterostrophus* is a recessive character, probably based on several genes. The fact that the western region varieties all had a relatively high resistance to this leaf blight would tally with the fact that there is little trade of maize into the area. The influx of seed into the eastern region could then explain the higher susceptibility of the maize varieties in the wedge, and the low number of cases of 'resistant' varieties found in Northern Nigeria, in spite of the fact that the numerous 'Southern' inhabitants of the northern region have, doubtlessly, taken southern maizes to the north, would then be understandable.

C. L. M. van Eijnatten

Botany Division,
Federal Agricultural Research,
Moor Plantation,
Ibadan, Nigeria.

[1] Bunting, R. H., *Gold Coast Dept. Agric. Bull.* 10 (Govt. Printer: Accra, 1928).
[2] West, J., *Kew Bull.*, 1 (1938).
[3] Stanton, W. R., West African Maize Research Unit, Second Ann. Rep. (Moor Plantation, Ibadan, Nigeria, 1954).
[4] *Report on the Sample Census of Agriculture 1950/51* (Department of Statistics, Survey Department, Lagos, 1952).
[5] Prest, A. R., and Stewart, I. G., *The National Income of Nigeria 1950/51* (Univ. of Cambridge, Department of Applied Economics, 1951)

Diurnal Variation in Transaminase Activity in the Cactus Plant

The rhythmic acidification and deacidification occurring in cactus and other succulent plants may be expected to be associated with rhythmic alteration in the activity of enzymes involved in the formation and transformation of the organic acids. A change in the activity of an enzyme may be envisaged as being brought about by altering the total amount of catalytic site available, that is, by the synthesis or destruction of enzyme protein, or by the elaboration or removal of activator or inhibitor molecules from the site of enzyme action, or by the accumulation of products of reaction. It has been reported from this laboratory[1] that cactus plants growing in garden soil in the open showed a marked diurnal variation in aldolase activity and it was suggested that the peak of its activity, which was reached in the dark, was linked with the breakdown of carbohydrate to the 3-carbon atom acceptor of carbon dioxide. Continuing the above investigations, we have found that the phylloclades of *Nopalea dejecta* grown in the open under natural conditions of light and darkness show a remarkable diurnal variation in glutamic oxaloacetic transaminase activity.

The transaminase activity was determined by the colorimetric method of Tonhazy *et al.*[2] on homogenates of pooled samples of phylloclades collected at 4-hourly intervals over a 24-hr. period in February and in March of this year. A unit of enzyme was defined as the amount which gave rise to 1 μgm. of pyruvic acid at 30° C. in 30 min. at *p*H 7·4. Specific activity of the enzyme was calculated as units per mgm. dry weight of tissue. Protein was estimated by the biuret method of Gornall *et al.*[3] after precipitation with trichloracetic acid, and total nitrogen by the micro-Kjeldahl method. In some experiments the non-protein nitrogen was determined by Kjeldahl digestion of the centrifuged supernatant from the precipitate formed with trichloracetic acid.

The results reported in Table 1 show that the transaminase activity was maximum at 4 p.m. and was markedly reduced at 8 p.m., to be followed by a further abrupt drop. The enzyme was thereafter maintained at an extremely low level, until noon, when the activity began to increase. When equal volumes of the homogenates from material collected at 4 p.m. and at 8 a.m. were mixed, it was found that the enzymic activity of the mixed homogenate was almost exactly the arithmetic mean of the individual activities of the two samples. This indicated that the observed diurnal alteration in enzymic activity was due essentially to changes in the quantity of active enzyme and not to the presence of activator or inhibitor. There was a rhythmic alteration also in the protein content, which, however, was much less pronounced than that of transaminase activity. Calculated on the basis of dry weight of tissue, the protein content was high in the day-time and low during the night.

Saltman *et al.*[4] demonstrated that during the exposure of leaves of *Bryophyllum calycinum* to

Table 1. Diurnal Variation in Transaminase Activity in *Nopalea dejecta*

Time of collection of samples	Temperature (°C.)		Dry weight, mgm./ml. 40 per cent homogenate	Specific activity (units/mgm. dry weight)	Acidity, ml. *N*/10 sodium hydroxide/gm. dry weight of tissue	Protein, mgm./gm. dry weight of tissue
	Ambient	Inside phyllo-clade				
February 25, 1961						
8 a.m.	16·5	11·5	18·4	0·48	26·1	80·5
Noon	26·0	29·5	20·5	8·48	14·6	104·8
4 p.m.	26·0	26·5	17·9	25·84	5·6	88·9(?)
8 p.m.	18·0	13·5	19·4	12·76	8·3	105·0
12 p.m.	16·0	12·0	19·2	10·59	11·5	92·6
4 a.m.	12·5	10·0	22·8	3·62	13·2	66·6
March 11, 1961						
8 a.m.	23·0	17·5	17·4	0·51	23·0	85·1
Noon	34·0	40·0	21·7	2·99	12·9	102·4
4 p.m.	35·0	30·5	20·8	20·83	5·8	122·8
8 p.m.	20·0	20·0	20·6	13·45	7·8	106·1
12 p.m.	21·0	18·5	20·0	3·68	12·0	85·2
4 a.m.	17·5	16·5	18·5	1·12	18·4	62·1

labelled carbon dioxide in the dark for 1 min.–2 hr., radioactivity was incorporated into the acids of the Krebs tricarboxylic acid cycle and amino-acids such as glutamic acid, aspartic acid and alanine. The authors considered that the amino-acids arose from the α-keto acids of the cycle by transamination reactions. The extremely low activity of transaminase observed by us at night may be indicative of predominance of organic acid production during dark fixation of carbon dioxide in cactus, with minimal transformation of α-keto acids to amino-acids. Conversely, the high activity of the enzyme commencing at noon and reaching an abrupt peak at 4 p.m. may be associated with increased amino-acid synthesis in the day-time. Our work on non-protein nitrogen of cactus tissue has been of a preliminary nature, but estimations of total protein reveal a rhythmic increase in the day-time.

One of us (S. K. M.) thanks the Ministry of Scientific Research and Cultural Affairs, Government of India, for the award of a Senior Research Training Scholarship.

S. K. Mukerji
G. G. Sanwal
P. S. Krishnan

Division of Biochemistry,
University of Lucknow,
Lucknow, U.P.

[1] Sanwal, G. G., and Krishnan, P. S., *Nature*, **188**, 664 (1960).
[2] Tonhazy, N. E., White, N. G., and Umbreit, W. W., *Arch. Biochem.*, **28**, 36 (1950).
[3] Gornall, A. G., Bardawill, C. S., and David, M. M., *J. Biol. Chem.*, **177**, 751 (1949).
[4] Saltman, P., Lynch, V. H., Kunitake, G. M., Stitt, C., and Spolter, H., *Plant Physiol.*, **32**, 197 (1957).

Shortening the Juvenile Phase of Apple Seedlings

Apple seedlings usually take 7–14 yr. to come into bearing. Many attempts have been made by plant breeders and others to reduce this juvenile phase, in order to obtain an earlier assessment of new seedlings.

Variations in pruning, nutrition and cultural practices have failed to shorten this juvenile period. By working seedlings on the dwarfing rootstock *MIX*, Tydeman[1] was able to shorten the juvenile phase by approximately one year. This practice has the disadvantage that many clones of this rootstock carry virus diseases and will consequently infect the new seedlings. It is therefore necessary to maintain the unworked seedling as well as its progeny worked on the rootstock, a system which doubles the land required by the plant breeder.

It has been found that apple seedlings worked on apomictic seedling rootstocks from *Malus sikkimensis* have a shorter juvenile phase than when worked on *MIX*; and since the apomictic seedlings are virus-free it should not be necessary to maintain the original seedling.

In an experiment begun in 1956, three crosses were made from which twenty-five seedlings were raised. These were multiplied vegetatively during their first year by budding on to one-year-old seedlings of *M. sikkimensis*; the young trees were later transplanted into the orchard.

It can be seen (Table 1) that 15 per cent of the trees were flowering 3 yr. after budding and 53 per cent after 4 yr. Since about one-third of the trees were fruiting by the end of their fourth year, and were carrying an average of seven fruits, it was possible to obtain an early assessment of the value of these new seedlings. None of the original twenty-five seedlings planted alongside for comparison has fruited during the same period, although both sets of trees have been treated similarly and no pruning has been carried out.

The possibility that other apomictic species may have a similar effect on the juvenile phase of apple seedlings worked on them is being investigated.

A. I. Campbell

Department of Agriculture and
Horticulture,
University of Bristol,
Research Station, Long Ashton.

[1] Tydeman, H. M., *Ann. Rep. East Malling Res. Sta. for 1927*, 51 (1928).

Cactus Virus in the United States

Since the first report by Molisch[1] of the presence of proteinaceous spindles in the cells of certain cacti, a series of researches has been made demonstrating the association of virus with the spindles and with amorphous inclusions[2].

The immediate source material for these investigations has been exclusively from European greenhouses and botanical gardens. We can now report the presence of virus in cacti cultivated in the United States.

Characteristic cigar-shaped spindles, usually one, but occasionally two to the cell, have been observed, mainly in the hypodermal cells of free-hand sections of pads of *Opuntia monacantha f. variegata* and of four other kinds of flat-padded opuntias cultivated in Montana and California. We examined one field sample of *O. lindheimeri* shortly after it was collected in Texas. No inclusions associated with virus were

Table 1. Apple Seedlings worked on *Malus sikkimensis*

Family	No. of trees	Third year				Fourth year			
		Per cent flowering	Per cent fruiting	Av. No. per tree		Per cent flowering	Per cent fruiting	Av. No. per tree	
				Flower clusters	Fruits			Flower clusters	Fruits
Newton Wonder × Emneth Early	88	23	6	4	7	57	25	17	18
Newton Wonder × Woolbrook Pippin	86	5	2	6	1	45	37	32	7
Newton Wonder × Mr. Prothero	34	20	19	4	2	38	29	41	

found. Such inclusions were reported previously to be present in a cultivated sample of this species[2]. No spindles have been found in pads of twenty cactus species examined shortly after field collection in Arizona, California and Montana.

In all cases, virus transmission was successful, as judged by the development of spindles, when sap from spindle-containing specimens was injected into or rubbed on the previously spindle-free pads of *Opuntia* species growing in the Montana State University greenhouse and the University of California at Los Angeles Botanical Garden.

No external symptoms appeared when representatives from the families Leguminosae, Solanaceae, Chenopodiaceae, Cruciferae, Tropaeolaceae or Cucurbitaceae were inoculated. Electron micrographs made from spindle-containing pads by the quick-dip method[3] showed two groups of elongated particles, with one length peak at 515 mμ (similar to cactus virus 1 (ref. 4)) and another at 300 mμ.

We wish to thank H. O. Agrawal and R. A. Solberg for help with some experiments, and Dr. L. Bos for the electron microscopy. This work was supported by the U.S. Public Health Service Grant No. *E*-596(*c*).

(The late) Irene M. Sammons
M. Chessin

Department of Botany,
Montana State University,
Missoula, Montana.

[1] Molisch, H., *Ber. Deutsch. Bot. Ges.*, **3**, 195 (1885).
[2] Amelunxen, F., *Protoplasma*, **49**, 140 (1958).
[3] Brandes, J., *Nachrbl. deut. Pflanzenschutzdienst* (Braunschweig), **9**, 151 (1957).
[4] Brandes, J., and Wetter, C., *Virology*, **8**, 99 (1959).

Antimicrobial Substances from Ferns

It has been almost twenty years since Osborn[1] reported his now classical work on antimicrobial substances from green plants. Since that time the literature accumulated on this subject has been voluminous[2]. The vascular plants tested, however, have been essentially spermatophytes, with little attention to the pteridophytes. This work is concerned with the extraction of antimicrobial substances from thirty ferns collected at the Brooklyn Botanical Gardens. The specimens were selected at random, and the cut fronds were allowed to dry in air for at least one week before testing. Four additional dried ferns were obtained from other sources and likewise tested.

Extracts of the plants were prepared in the following manner: 15 gm. of pulverized material were added to 100 ml. of methanol and macerated in a Waring blendor for 3 min. and filtered. Filter-paper disks (6·35 mm. diam.) were saturated with the extract and allowed to dry for 3 hr. The surface of nutrient agar dishes was seeded with 0·5 ml. of a 48 hr. bacterial broth culture while 0·5 ml. of a one-week-old fungal culture was used to seed Sabauraud maltose agar dishes. The prepared disks were placed on the surface of the seeded dishes and incubated. All micro-organisms were incubated for 48 hr., the bacteria at 37° C., the fungi and *Erwinia caratovora* at 22° C. The zones of inhibition were measured from the disk edge to the zone edge and recorded in mm.

In Table 1 is listed the ferns and their zones of inhibition against 8 bacteria. No zones of inhibition were produced against *Aspergillus niger* ATCC 6277, *Candida albicans* ATCC 10231, *Gibberella fugikuroi* NRRL 2284, and *Helminthosporium trucicum* ATCC 11535. The results indicate that antibacterial substances were extracted in the methanol-soluble fraction from 33 of the 34 specimens tested and that plant pathogenic bacteria are more susceptible than human pathogens. This may perhaps explain the relative resistance of ferns to bacterial invaders. Additional extracts are being prepared in this Laboratory and will be the subject of future reports.

Table 1. Antibacterial Properties of Ferns

	11766	8061	10692	9637	9491	9372	8245	9592*
Cyatheaceae								
Alsophila cooperi	+	−	−	−	−	−	−	−
Cibotium glaucum	+	−	+	−	−	+	−	−
Cibotium menziesii	+	+	−	−	+	+	−	−
Cibotium schiedei	+	+	−	−	+	+	−	+
Marrattiaceae								
Angiopteris evicta	+	−	−	−	+	+	−	−
Polypodiaceae								
Acrostichum aureum	−	−	−	−	−	−	−	−
Adiantum capillus-veneris	+	+	−	−	−	+	−	+
Adiantum trapeziforme	+	−	+	−	−	−	−	−
Asplenium nidus	+	−	+	−	−	+	−	+
Blechnum spicant	+	−	−	−	−	+	−	−
Cyrtomium falcatum	+	−	−	−	−	−	−	−
Davallia pentaphylla	−	+	−	−	−	−	−	+
Dennstaedtia punctilobula	+	−	+	+	−	+	−	−
Doodia media	+	−	+	−	−	−	−	−
Dryopteris dentata	+	−	−	−	+	−	−	−
Dryopteris filix-mas†	+ + +	−	− −	−	+ +	+ −	− −	+
Microlepia strigosa	+	+	+	−	−	−	−	−
Nephrolepis ensifolia	+	−	+	+	+	−	−	−
Nephrolepis exaltata, a variety	+	−	−	−	−	−	−	−
Polypodium aureus	+	−	−	−	−	+	−	−
Polypodium Meyenianum	+	−	−	−	−	+	−	−
Polypodium punctatum	+	−	+	+	+	+	−	−
Polypodium quercifolia	+	−	+	−	−	−	−	−
Polystichum tsus-sinense	+	−	−	−	+	+	−	−
Pteridium aquilinum	−	+	−	−	−	−	−	−
Pteridium aquilinum†	−	+	−	−	−	−	−	−
Pteris tremula	−	+	−	−	+	+	−	−
Pteris vittata	+	−	−	−	+	−	−	−
Pyrrosia lingua	+	−	−	−	−	−	−	−
Stenochlaena tenuifolia	+	−	−	−	−	−	−	−
Tectaria cicutaria	+	−	−	−	−	−	−	−
Tectaria incisa	+	−	−	−	−	−	−	−
Thelypteris setigeria	+	+	−	−	+	−	−	−
Schizaeaceae								
Lygodium japonicum	+	−	+	−	+	+	−	−

* Bacterial test organisms: *Xanthomonas phaseoli* var. *sojensis* ATCC 11766, *Erwinia caratovora* ATCC 8061, *Pseudomonas solanacearum* ATCC 10692, *Escherichia coli* ATCC 9637, *Staphylococcus aureus* ATCC 9491, *Bacillus subtilis* var. *niger* ATCC 9372, *Bacillus megaterium* ATCC 8245, *Bacillus cereus* ATCC 9592.
† Rhizones and roots.
+, Zones of inhibition 1–5 mm.; + +, zones 6–10 mm.; + + +, zones 11–15 mm.; −, zone of inhibition absent.
Each extract was tested at least in triplicate.

Jasper C. Maruzzella

Biology Department,
Long Island University,
Brooklyn, New York.

[1] Osborn, E. M., *Brit. J. Exp. Pathol.*, **24**, 227 (1943).
[2] Nickell, G. L., *Econ. Bot.*, **13**, 281 (1959).

Introduction of the Potato into Western and Central Europe

The botanist Clusius, who prepared one of the first descriptions of the potato plant[1], indicated not only the time (the beginning of 1588) of his receiving from Philippe de Sivry (Prefect of the City of Mons, Belgium) two potato tubers and a berry, but also when Philippe de Sivry himself obtained the tubers. Clusius wrote that Philippe de Sivry had received it in the preceding year from a certain friend of the Papal Legate in Belgium under the name of 'Taratouffli'. ("Is a familiari quodam Legati Pontifici in Belgio se

acceptisse scribebat, anno praecedente Taratouffli nomine"). Roze, the author of *Histoire de la Pomme de Terre*[2], translating into French Clusius's description, erroneously omitted the words "in the preceding year". The same mistake was repeated by Redcliffe Salaman in his monograph *The History and Social Influence of the Potato*[3]. Fuess[4] in Germany, as a result of Roze's fault and evidently having no original text by Clusius, erroneously supposed the time of potato introduction into Mons to be the year 1566. Roze admitted the second mistake in the French translation of Clusius's potato diagnosis. Clusius describes the shape of his potato berries as "orbiculata". That strictly means not globose or rounded but disk-shaped, and his description is very true to the picture. In Salaman's English translation of Clusius's diagnosis, the same lack of punctiliousness is observed.

Disk-shaped berries are rare to-day among cultivated potatoes, and this morphological feature, as well as some other ones (namely, numerous stolons and tubers, very different tuber size, rather late tuberization, probably deep eyes, weakly dissected leaves with short leaflet stalks, leaflets without elongated apices, numerous blossoms, calyx sharply limited from the top of the pedicels, according to the picture probably spear-leaved lobes of the calyx, deeply dissected calyx and corolla, green rays of stella, evidently short anthers (in broad cone ?) and odoriferous flowers), lead me to suppose that at least one tuber of two, or a plant from the berry which Clusius received in Vienna from Philippe de Sivry, belongs to the circle of forms *S. andigenum* Juz. et Buk. convarietas curtibaccata Lechnovitch, from South Peru. The present-day German potato (var. Jubel) has the same type of berries, anthers, and leaves and is, perhaps, connected with Clusius's material. The date of at least one, probably the earliest and possibly the chief, introduction of the potato into Western and Central Europe is quite clear.

V. S. Lekhnovitch

All-Union Crop Husbandry
Research Institute,
44 Herzen Street,
Leningrad, 1.

[1] Clusius, Carolus, *Rariorum plantarum historia* (Antverpiae, 1601).
[2] Roze, E., *Histoire de la Pomme de Terre* (Paris, 1898).
[3] Salaman, R. N., *The History and Social Influence of the Potato*, p. 89 (Cambridge, 1949).
[4] Fuess, W., *Die Urheimat der Kartoffel, ihre Einführung und Ausbreitung in Europa. Ernähr. d. Pflanze*, **31** (17), 288 (1935).

ENTOMOLOGY

Air-Swallowing : a Mechanism in Photic Reversal of the Beetle *Trypodendron*

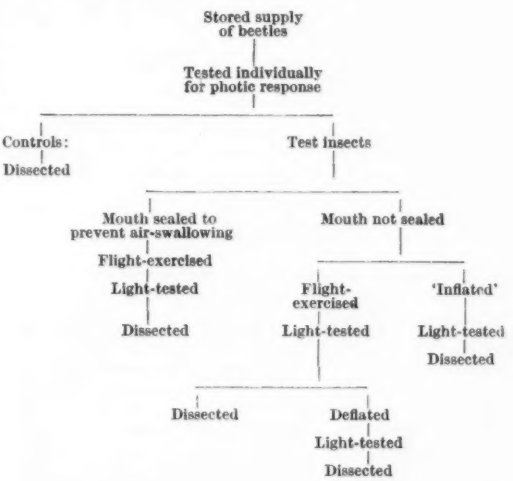
A PREVIOUS communication[1] reported the effectiveness of flight exercise in abolishing the overruling response to light in overwintered adults of the ambrosia beetle *Trypodendron* (*Xyloterus*) *lineatum* Olivier. This observation is ecologically significant in explaining the behaviour of the insect in terms of one of its normal experiences. Evidently the photopositive response which initially compels it, advantageously, to dispersive flight indirectly sponsors termination of flight and unmasks a latent responsiveness to host factors, allowing it to settle down.

The change seemed to occur only after a period of flight, never in its absence. Nevertheless, belief in the role of flight could be strengthened if a possible mechanism could be demonstrated. Furthermore, a demonstrable mechanism might aid in the interpretation of anomalous behaviour in other work with this insect, or be used for experimental purposes.

Some of the more obvious facts, drawn from existing knowledge, were brought to bear on the question. Chapman[2] had reported, for the same species, that the ventriculus, and often the œsophagus, of beetles taken in flight are distended by a large gas bubble. The significance of the discovery was not clear at the time.

The coincidence of the two phenomena, namely, photic reversal and gas accumulation, associated with a common experience, namely, flight, could not be ignored, but how could gas in the alimentary tract be assumed to affect behaviour ? The first and simplest possibility was that it exerts pressure and creates a signal that is relayed to the central nervous system. Photic reversal associated with pressure in the body of spruce budworm larvæ had been observed by Wellington[3]. The reversal occurred regardless of how the pressure was applied, whether from food, injected fluid or externally applied pressure of a ligature. The influence of pressure is relayed to the higher neural centres through the 5th abdominal ganglion, which can be 'blocked' by local anæsthetizing with methylal (dimethoxymethane).

A schedule of tests was drawn up to compare responses of *Trypodendron* after various treatments contrived to introduce gas pressure.



Photic response was tested under room conditions at 20–21° C. and relative humidity ranging about 60 per cent. Beetles were placed on a paper surface to observe and trace with a pencil their orientation towards a window source of light. Each beetle was given five trials beginning each time from a different direction of point relative to the light.

Beetles of both sexes fresh from overwintering, and thus non-flown, were photopositive in varying degrees. The rate of turn towards light, rate of crawling, and frequency of attempts to fly varied with individuals. Some individuals took short (5–10 cm.) backward flights before taking the typical laterally oscillating course, which advanced them at about 2 m./sec.

Unflown beetles generally had no gas bubble in the ventriculus, and rarely had a small one.

Beetles with a viscous seal over the mouth did not change behaviour after flight, and dissection revealed no gas bubble.

Beetles suspended by the pronotum responded variously to the opportunity for anchored flight, some maintaining almost uninterrupted wing movement for hours, others being intermittent, and still others 'flew' only occasionally. Prolonged flight was generally followed by a decisive reversal of photic response, and distension of the gut with gas was always associated with photic reversal. Deflation by piercing through the elytra and soft dorsum into the alimentary tract with a finely drawn glass capillary (when not accompanied by surgical damage and paralysis) restored the photically reversed beetles to light-positive behaviour.

Unflown beetles, artificially inflated by blowing through a fine drawn capillary sealed to the mouth of the beetle with heavy grease, became photically reversed. Dissection confirmed the successful inflation.

The evidence is thus: that the unflown beetles are strongly photopositive; that through prolonged flight, but not without it, they become indifferent or negative to light; that accumulation of gas in the gut accompanies the change, and the change is always associated with the gas; that deflation restores the photopositive response; and that artificial inflation is effective in abolishing the photopositive response.

The evidence is thus reasonably conclusive that the release of responses other than photic in *Trypodendron* is brought about by air swallowed during flight, acting presumably by its pressure.

Kenneth Graham

Department of Zoology,
University of British Columbia,
Vancouver 8.

[1] Graham, K., *Nature*, **184**, 283 (1959).
[2] Chapman, J., *Proc. Tenth Intern. Congr. Ent.*, **4**, 375 (1956, 1958).
[3] Wellington, W. G., *Canad. Entomol.*, **80**, 56 (1948).

MICROBIOLOGY

Oxygen Inhibition of Respiration in *Azotobacter*

The effect of oxygen partial pressure on *Azotobacter* has been studied by Meyerhof and Burk[1], Burk[2] and by Parker and Scutt[3], with essentially similar findings. Respiration in this organism reaches a clearly defined maximum at 0·15–0·30 atm. oxygen, Burk's results tending to the lower values and Parker and Scutt's to the higher. Respiration was measured with either sucrose or glucose as the substrate, and a similar observation was made by Tschapek and Giambiagi[4], who followed glucose dissimilation rather than oxygen uptake. Inhibition of some enzyme system(s) in the respiratory mechanism appears to be the most likely explanation of this effect.

The oxygen effect seems to be peculiar to *Azotobacter*, since we were unable to find such a well-defined inhibition when the respiration of *Bacillus subtilis* (N.C.T.C. 518), *Saccharomyces cerevisiae* and *Rhizobium trifolii* (Australian strain *NA* 30) was measured on sucrose as substrate under different oxygen partial pressures ranging from 0·05 atm. to 1·00 atm. oxygen (Fig. 1).

An attempt has been made to identify a stage sensitive to oxygen of the respiratory system using various substrates as the carbon source and testing for the presence of the inhibition.

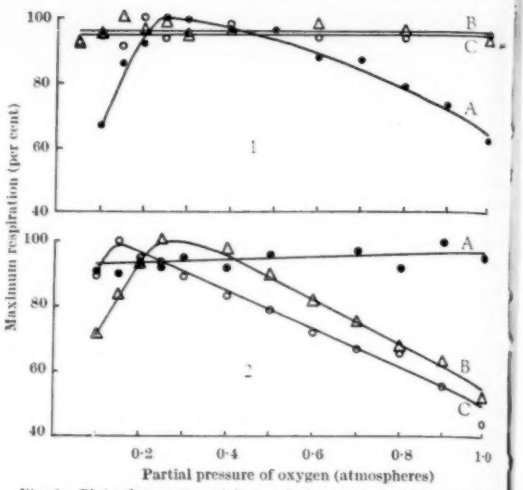


Fig. 1. Plot of oxygen uptake against oxygen partial pressure for three different organisms. Values indicate the percentage of the maximum uptake observed. Curve *A*, *Azotobacter vinelandii*, strain Wisconsin *O*. Curve *B*, *Rhizobium trifolii*, strain *NA* 30. Curve *C*, *Bacillus subtilis*, N.C.T.C. 518

Fig. 2. Plot of oxygen uptake against oxygen partial pressure for *Azotobacter vinelandii* on different substrates. Curve *A*, glycerol. Curve *B*, pyruvate. Curve *C*, succinate

Cells of *A. vinelandii* (strain *O*, kindly supplied by Prof. P. W. Wilson) were grown overnight on synthetic media, using sucrose and urea as the carbon and nitrogen sources, respectively. The cells were recovered by centrifugation, re-suspended in a complete mineral salts medium containing the desired carbon source, and aerated for 4 hr. in the presence of urea as a source of nitrogen. This period sufficed for adaptation of the cells[5]. Oxygen uptake was then measured by the conventional Warburg technique in short-term experiments ($\frac{3}{4}$ hr.), using a known density of organisms under oxygen–argon mixtures containing 0·1–1·0 atm. oxygen.

Comparable results to those given with sucrose were found for glucose, pyruvate, acetate, succinate and malate, although the inhibition was more severe with the organic acids than with the sugars. Sample curves for three of these substrates are shown in Fig. 2. Glycerol is the obvious exception, no inhibitory effects of oxygen being detectable in this case.

Addition of —SH group stabilizing reagents (cysteine, glutathione) at concentrations up to 0·01 *M* failed to protect the organisms from the toxic effects of oxygen. That they are permeable to cysteine was shown by the toxic effect of the higher levels of cysteine used.

The failure of the organic acids to 'short-circuit' the inhibition suggests that the oxygen-sensitive system must be external to glycolysis, lying either in a tricarboxylic acid cycle known for these organisms[6], or in the terminal respiratory pathway. Inhibition by oxygen in the former cycle has been reported for pyruvic acid oxidation in *Staphylococcus aureus*[7] and rat brain[8], and for a step in the reactions between citrate and α-oxoglutarate in potato tubers[9]. Oxygen inhibition of an enzyme in the latter pathway has been shown recently for cytochrome *c* reductase[10].

It is postulated that the pattern seen in Fig. 1 for *A. vinelandii* occurs as a result of two opposing systems: (*a*) a terminal oxidation system which reaches a maximum value about 0·25 atm. oxygen

and then maintains this value at least up to 1·0 atm. ; and (*b*) an oxygen-sensitive system the inactivation of which increases with increasing partial pressure of oxygen and which determines whether the potential activity of system (*a*) is reached.

The rising part of the experimental curve could then be explained as a rapid rise in the activity of (*a*) compared to which inactivation of (*b*) is negligible. At higher partial pressure of oxygen, the declining activity is due to inactivation of (*b*).

Examples known for system (*b*) have already been mentioned. A cytochrome oxidase system with characteristics of system (*a*) is known from *Pseudomonas*[11].

We are unable to offer an explanation of the apparent lack of inhibitory effect when glycerol is used as the substrate. It suggests the possibility that a permease system is being poisoned, but this seems unlikely as the oxygen effect is similar with substrates of neutral sugars or anionic organic acids. Further work on oxygen poisoning is in progress.

This work was carried out while one of us (M. J. D.) was in receipt of a Commonwealth Scientific and Industrial Research Organization Studentship, and later an I.C.I. (Australia and New Zealand) Research Fellowship. Grateful acknowledgment is made to these sources and to the University of Western Australia for research grants.

M. J. Dilworth
C. A. Parker

Soil Microbiology Section,
Institute of Agriculture,
University of Western Australia,
Nedlands.

[1] Meyerhof, O., and Burk, D., *Z. physik. Chem.*, A, **139**, 117 (1928).
[2] Burk, D., *J. Phys. Chem.*, **34**, 1195 (1930).
[3] Parker, C. A., and Scutt, P. B., *Biochim. Biophys. Acta*, **38**, 230 (1960).
[4] Tschapek, M., and Giambiagi, N., *Arch. Mikrobiol.*, **21**, 376 (1955).
[5] Williams, A. M., and Wilson, P. W., *J. Bact.*, **67**, 353 (1954).
[6] Stone, R. W., and Wilson, P. W., *J. Bact.*, **63**, 605 (1952).
[7] Wolin, M. J., Evans, J. B., and Niven, C. F., *Arch. Biochem. Biophys.*, **58**, 356 (1955).
[8] Mann, P. J. G., and Quastel, J. H., *Biochem. J.*, **40**, 139 (1946).
[9] Barker, J., and Mapson, L. W., *Proc. Roy. Soc.*, B, **143**, 523 (1955).
[10] Dixon, M., Maynard, J. M., and Morrow, P. F. W., *Nature*, **186**, 1032 (1960).
[11] Horio, T., Higashi, T., Matsubara, H., Kusai, K., Nakai, M., and Okunuki, K., *Biochim. Biophys. Acta*, **29**, 297 (1958).

A Micro-method for performing the Phenylalanine Test on Cultures from Multi-carbohydrate Media

The phenylalanine test is of great value for investigating cultures of organisms in the family Enterobacteriaceae. Organisms in the *Proteus* group are able to de-aminate the amino-acid, phenylalanine, to form phenylpyruvic acid, whereas members of the other groups in the family are unable to do so. Buttiaux *et al.*[1] claim that this test is more reliable than the urease test for the identification of *Proteus* strains.

In a previous communication[2] a technique was described in which the bacterial growth from Kligler's iron agar, or other multiple carbohydrate media, could be tested for its ability to de-aminate phenylalanine. Although this technique gives reliable results, it has several disadvantages : (1) the removal of the cells from the Kligler's iron agar slope by washing with phenylalanine solution leaves no cells for further biochemical or serological examination ; (2) a shaker and bijou bottles (which need to be sterilized and cleaned before re-use) are needed ; (3) it is time-consuming to test a large number of cultures ; (4) the cost of the phenylalanine reagent and of the glassware preparation is not inconsiderable.

With these points in mind, a much simpler technique was evolved, whereby the original bacterial culture on Kligler's medium was retained, neither a shaker nor bijou bottles were required and the cost of performing the test was reduced by more than 90 per cent.

In the micro-method now proposed, ordinary 3 in. × 1 in. glass slides have six squares ($\frac{7}{8}$ in. × $\frac{3}{8}$ in. or 1 cm. × 1 cm.) etched on to them on one side, with a diamond glass cutter. The slides are then warmed in a bunsen burner flame, and a black wax pencil is rubbed along the etched lines, leaving these clearly marked in black, when the excess wax is rubbed off with a soft cloth.

From a Pasteur pipette, 1 drop of a 0·4 per cent β-L-phenylalanine solution prepared in 0·01 *M* phosphate buffer at *p*H 7·4 is placed in the centre of each of the etched squares on the slides. A heavy inoculum of bacterial culture is removed from the slope of the Kligler's medium, using a loop, and this growth is emulsified in one of the drops of phenylalanine. This is best done holding the slide against a black background, as it is essential to obtain as heavy a cell suspension as possible. The waxed, etched lines prevent the drop from spreading beyond its square (Fig. 1).

The slide is now placed on a circle of damp filter paper in the bottom of a Petri dish, and, after the cover has been replaced, the assembly placed for 20 min. at 37° C. in an incubator. At the end of this period, 1 drop of an 8 per cent ferric chloride solution (aqueous) is added to each of the drops of bacterial suspension on the slide, and the Petri dish is tapped gently on the side to aid the mixing of the drops. An immediate dark green colour develops if phenylpyruvic acid has been formed. This green colour fades within a minute, therefore the reactions should be noted at once.

In a comparison made between this micro-method and the method previously described[2], thirty cultures

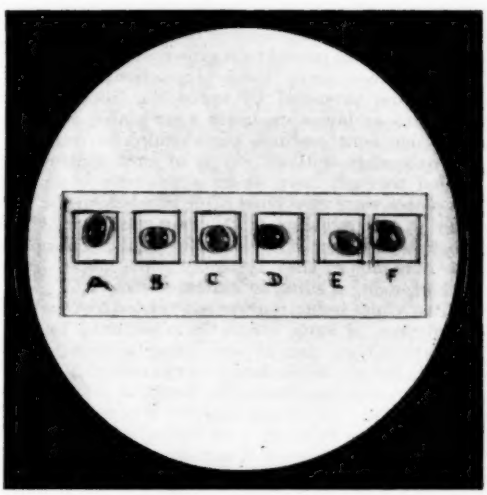


Fig. 1. Slide showing etched, waxed squares, and phenylalanine tests with cultures marked *A*, *B*, *C*, *D*, *E* and *F*. Only *D* is showing a positive reaction

of organisms in the Enterobacteriaceae, including twenty *Proteus* strains, were inoculated on to Kligler's iron agar, incubated for 18–24 hr. at 37° C., and both methods of performing the phenylalanine test were used on the bacterial growth from the slopes. In all cases the micro-method gave the same results as the other method; all the *Proteus* strains were strongly positive, all the other strains were negative (yielding yellow or brown colorations).

Similarly, using another multiple carbohydrate medium: phenol red agar base (Difco) 3·1 per cent, lactose 1 per cent, sucrose 1 per cent and mannitol 0·1 per cent, identical results were obtained.

After completing the micro-test, the slides may be placed in a disinfectant solution until required again, when all they need is to be rinsed with running water and dried.

This simple micro-technique would seem to allow the rapid examination of large numbers of cultures such as might be encountered in the routine and in the research laboratory.

D. J. Stewart

Ministry of Agriculture
for Northern Ireland and
The Queen's University of Belfast.

[1] Buttiaux, R., Moriamez, J., and Papavassiliou, J., *Ann. Inst. Pasteur*, **90**, 133 (1956).
[2] Stewart, D. J., *Nature*, **183**, 1537 (1959).

Absence of Lysogeny in *Pasteurella pestis*

A survey of the literature suggests that most bacteria are lysogenic for one or more phages. This communication reports failure to detect or establish lysogeny among strains of *Pasteurella pestis*. The object of the work was to obtain a temperate phage suitable for genetic study by means of transduction[1] of factors determining virulence in this organism[2]. Fifty-four different strains of *P. pestis* (Table 1) were screened for lysogeny and attempts were made to establish lysogeny with phages lytic for *P. pestis*.

In the screening experiments, a method based on that of Fisk[3] was used. Each of the 54 strains was tested both for lysogeny and as a potential phage indicator. Organisms derived from overnight cultures in tryptic digest meat broth grown at 28° C. in gently rotated tubes were used throughout. Indicator plates were prepared by spreading about 2×10^8 organisms as lawns on broth agar plates containing 0·075 per cent sodium metabisulphite and dried. Approximately 0·01-ml. drops of each culture were applied to each lawn using a multiple inoculator[4]. The plates were examined after incubation for 40 hr. at 28° C. No evidence of lysogeny was seen. Known lysogenic strains of *Salmonella typhimurium* gave clear evidence of their lysogeny when tested by this method, using a suitable indicator strain.

Ultra-violet induction was attempted by irradiation of cultures of each strain in phosphate buffer at 9 ergs/mm.²/sec. for 20 sec. using a Hanovia low-pressure lamp. None showed a reduction in turbidity on subsequent incubation in broth at 28° C. over a 10-hr. period. No phage activity was detected when drops of these cultures were plated on lawns of the 54 strains of *P. pestis*, 5 strains of *P. septica*, 5 of *P. haemolytica* or 6 of *P. pseudotuberculosis*.

In case failure to detect lysogeny had been due to the spontaneous liberation of small numbers of phage particles only by a strain of *P. pestis*, two mixed cultures consisting of about 5×10^7 organisms of all strains were shaken at 28° C. and 37° C. respectively for 6 hr. to permit such phage, if present, to propagate on a sensitive strain and so increase the chance of its detection. The mixed cultures were centrifuged and the supernatants sterilized with chloroform. None of the *P. pestis* strains was lysed by these culture fluids. Since it was possible that strains of *P. pestis* failed to act as indicators because they were all lysogenic for the same or related phage, attempts were made to 'cure' a strain by repeated heavy ultra-violet irradiation[5]. However, derivatives of strains of *TS* which had been subjected to one, two, three or four serial irradiations in buffer at 11 ergs/mm.²/sec. for 3 min. used as potential phage indicators also failed to reveal phage in the same culture fluids.

Table 1. Sources of Strains of *Pasteurella pestis* Investigated

Microbiological Research Establishment, Porton, England	National Collection Type Cultures, Colindale, England	Dr. R. Devignat, Elisabethville, Congo
WCDC Pp1	*EV(M)*	*A16*
WCDC Pp6	*P.F.2*	*A28*
WCDC Pp54	Parel	*A29*
WCDC Pp5A 2258	*T.S.*	*A32*
New Mexico 499559	Bombay 1	*A35*
New Mexico 499668	Bombay 2	*A36*
I.72		*M*
14		*M1d*
Shasta		*M38*
EV76		*M39*
139L		*M40*
F7793/10		*O12*
F9581		*O13*
5341		*O14*
L27		*O15*
L36		*O18*
TRU		*O19*
Harbin		*O20*
Yokohama		*O23*
Java		*O24*
Tjiwidej *S (TS)*		*O25*
A1122		*O26*
Soemedang		*O27*
Elisabethville		
MP6		

Of 17 phages which were lytic for strains of *P. pestis* at 28° C. or 37° C. on sodium metabisulphite medium (8 isolated from local sewage and 9 donated preparations) one, *P1k* (ref. 6), gave completely turbid plaques and three others plaques with wide turbid edges. Isolates from the former gave only non-lysogenic colonies resistant to *P1k*: those from the latter gave sensitive bacteria and virulent phage which could apparently be 'carried' through at least one single-colony isolation. Microcolonies appearing on lysed lawns of *P. pestis* strains after incubation for 5–7 days at 28° C. followed by 14 days at room temperature also yielded only sensitive bacteria and 'carried' phage. On the basis of host range and efficiency of plating, at least 7 of the 17 phages appeared to be different. Had any of them arisen from the same temperate phage by different mutations to virulence, multiple infection with pairs of them could have resulted in the establishment of lysogeny[7]. This was attempted by inoculating separate lawns of strain *TS* on sodium metabisulphite medium infected with each of 15 of the phages with drops of suspensions of each of them using the multiple inoculator. The multiplicity of infection for both phages within each area covered by a drop was greater than one and the whole process took only 25 min. On prolonged incubation only slow-growing microcolonies appeared which were again shown to consist of sensitive bacteria and virulent phage.

If any strains of *P. pestis* are lysogenic they, or suitable indicator strains, or both, are rare. There

are a few reports of failure to detect lysogeny in other species of bacteria[8]. Of these, only Williams Smith's failure to detect it among 62 strains of *Salmonella pullorum* involved experiments on a similar scale to those with *P. pestis*. It is possible that all *P. pestis* strains tested are lysogenic for the same non-inducible phage. The occurrence of colonies comprising sensitive *P. pestis* organisms and virulent phage is thought to be a further example of 'pseudolysogeny'[9], although less stable than that previously reported for this organism[10].

I am indebted to Dr. R. Devignat of Elisabethville, Congo, for strains of *P. pestis*, and Drs. N. Boulgakov, G. Girard and F. Jacob, of Paris, and Dr. D. Eisler, of California, for phage preparations. Discussion with Dr. T. W. Burrows and the technical assistance of Miss R. M. King are gratefully acknowledged.

D. A. Smith*

Microbiological Research Establishment,
Porton,
Salisbury,
Wilts.

* Present address: Department of Microbiology, University of Birmingham, 15.

[1] Zinder, N. D., and Lederberg, J., *J. Bact.*, **64**, 679 (1952).
[2] Burrows, T. W., *Symp. Haffkine Inst., Bombay*, 14 (1960).
[3] Fisk, R. T., *J. Inf. Dis.*, **71**, 153 (1942).
[4] Smith, D. A., *J. App. Bact.* (in the press).
[5] Boyd, J. S. K., *Biol. Rev.*, **31**, 71 (1956). Zinder, N. D., *Virology*, **5**, 291 (1958).
[6] Lennox, E. S., *Virology*, **1**, 190 (1955).
[7] Levine, M., *Virology*, **3**, 22 (1957). Kaiser, A. D., *ibid.*, **3**, 42 (1957).
[8] McCloy, E. W., *J. Gen. Microbiol.*, **18**, 198 (1958). Russell, R. L., Jann, G. T., and Froman, S., *Bact. Proc.*, 39 (1958). Williams Smith, H., *J. Gen. Microbiol.*, **5**, 458 (1951).
[9] Jacob, F., Lwoff, A., Siminovitch, A., and Wollman, E., *Ann. Inst. Pasteur*, **84**, 222 (1953).
[10] Girard, G., *C.R. Soc. Biol., Paris*, **151**, 1068 (1957).

GENETICS

Effect of Hybridization on the Primary Polydipsic Trait of an Inbred Strain of Mice

A NON-OBLIGATORY polydipsia which is reflected in the excretion of large quantities of urine of low specific gravity has been found in more than 90 per cent of an inbred strain of mice (*STR/N*)[1-3]. It appears to be due to an innate thirst, the satisfaction of which is entirely unnecessary for survival. It is present in mild degree at the earliest ages studied (4 weeks), and is greatly accentuated at about 6 months of age. There is an associated high mortality in males due to the combined presence of polyuria and a urethral plug. The latter was found in almost all male mice of all strains examined, and is probably derived from seminal fluid.

To attempt to obtain information on the mode of inheritance of the trait, the intake of water and urine specific gravity[4] of F_1 and F_2 hybrids resulting from the mating of the inbred polydipsic strain and a non-polydipsic strain and back-crosses were studied at 1–5 months of age in a total of 250 animals. The mean urine specific gravities and intake of water of the hybrid mice tended to be intermediate between those for the polydipsic and non-polydipsic strains, but resembled much more closely the non-polydipsic strain values (Table 1). There was no consistent difference between F_1 and F_2 hybrids. For reasons which are not apparent, back-crosses with both polydipsic and non-polydipsic strains tended to resemble the polydipsic strain. The inheritance of the trait does not appear to be as a simple recessive. The precise mode of inheritance and the number of genes involved will require examination of considerably larger numbers of animals.

Emanuel Silverstein*

Laboratory of Pathology and
Histochemistry,
National Institute of Arthritis
and Metabolic Diseases,
National Institutes of Health,
Bethesda, Md.

* Present address: Department of Physiological Chemistry, University of Minnesota Medical School, Minneapolis 14, Minnesota.

[1] Silverstein, E., Sokoloff, L., Mickelsen, O., and Jay, jun., G. E., *Fed. Proc.*, **17**, 457 (1958).
[2] Silverstein, E., First International Congress of Endocrinology, Copenhagen, July 1960, *Periodica, Abstr.*, No. 61.
[3] Silverstein, E., Sokoloff, L., Mickelsen, O., and Jay, jun., G. E., *Amer. J. Path.*, **38**, 143 (1961).
[4] Silverstein, E., *J. App. Physiol.*, **16**, 194 (1961).

Chromosome Rearrangements induced by X-Rays in Immature Germ Cells of *Drosophila*

MANY investigations have indicated that few chromosomal aberrations or losses have been produced in the immature germ cells of *Drosophila*[1-3]. The present work deals with factors influencing the production of chromosome rearrangements by X-rays in early oocytes and oogonia of *Drosophila melanogaster*.

Attached-*X* females aged 2–3½ and 8–9½ days of the genetic constitution *sc ct*^{n} *oc car.y In* 49 *sn* and an *sc*^{V1}. *YS* chromosome were irradiated with 2,400 r. X-rays. The dose was given either in 2 min. or in two 1-min. fractions separated by an hour interval in air (*AAA*); or in two 1-min. fractions separated by an hour interval with a 53-minute nitrogen flushing of the flies (*ANA*). After treatment the females were mated with *y sc*^{S1} *B f In* 49 *v* males and periodically transferred for 32 days. The progeny were scored for detachments of the attached-*X*. Detachments involve translocations between the *X* and one of the following: the *Y*, the IVth or the major autosomes[4,5].

Table 1. URINE SPECIFIC GRAVITY AND WATER INTAKE OF *STR/N* AND *DBA/2JN* MICE AND THEIR F_1 HYBRIDS*

Strain	*STR/N*		*DBA/2JN*		(*STR/N* × *DBA/2JN*)F_1	
Sex	M	F	M	F	M	F
Number of animals	6	6	6	6	14	11
Body-wt. (gm.)	15 ± 2†	15 ± 2	14 ± 1	12 ± 1	14 ± 1	14 ± 1
Urine specific gravity	1·032 ± 0·006‡ (1·024–1·044)§	1·042 ± 0·004 (1·023–1·068)	1·072 ± 0·006 (1·061–1·081)	1·065 ± 0·008 (1·026–1·100)	1·065 ± 0·003 (1·027–1·105)	1·059 ± 0·005 (1·032–1·093)
Water intake†	9·2 ± 0·5‡	9·4 ± 1	7·2 ± 0·5	4·7 ± 0·6	5·4 ± 0·3	5·9 ± 0·3

* Animals 29–44 days old.
† Four daily water measurements made 20–28 days after urine specific gravity measurements, ml. per animal per 24 hr.
‡ *S.E.* § Range.

XUM

Table 1. Frequency of Detachments of Attached-*X* Chromosomes produced by Different Modes of Treatment

Days after irradiation	2–3½-day-old females: 2,400 r. No. except. / total females	*AAA** No. except. / total females	*ANA*† No. except. / total females	Control No. except. / total females	8–9½-day-old females: 2,400 r. No. except. / total females	*AAA** No. except. / total females	*ANA*† No. except. / total females	Control No. except. / total females
11–13	50 / 2,348 = 0·021	20 / 1,528 = 0·013	24 / 665 = 0·036	0 / 979	43 / 1,829 = 0·024	21 / 1,278 = 0·016	19 / 793 = 0·024	1 / 1,101
18–21	68 / 3,578 = 0·019	38 / 2,533 = 0·015	37 / 1,088 = 0·034	0 / 1,160	67 / 2,814 = 0·024	35 / 2,227 = 0·016	30 / 1,218 = 0·025	0 / 911
23–26	56 / 2,501 = 0·022	44 / 2,363 = 0·019	20 / 709 = 0·028	0 / 1,071	45 / 1,866 = 0·024	39 / 1,840 = 0·021	16 / 702 = 0·023	0 / 700
26–28	47 / 2,168 = 0·022	25 / 1,843 = 0·014	9 / 378 = 0·024	0 / 634	24 / 1,228 = 0·020	35 / 1,248 = 0·028	6 / 519 = 0·012	0 / 524
28–32	60 / 3,429 = 0·017	40 / 2,808 = 0·014	17 / 488 = 0·035	0 / 1,261	47 / 2,733 = 0·017	36 / 2,203 = 0·016	25 / 943 = 0·027	0 / 979
	Harmonically Weighted Frequencies of Exceptions for Each Treatment							
Days 11–32	0·020 ± 0·0012	0·015 ± 0·0012	0·032 ± 0·0031	0 / 5,105	0·022 ± 0·0014	0·019 ± 0·0015	0·023 ± 0·0018	1 / 4,215
0–4	0·049 ± 0·0030	0·040 ± 0·0027	0·072 ± 0·0068	0 / 4,927	0·050 ± 0·0038	0·052 ± 0·0040	0·086 ± 0·011	0 / 3,872

* *AAA* = air present during irradiations and air present during 1 hr. interval.

† *ANA* = air present during irradiations and nitrogen present during 53 min. of 1 hr. interval.

The results are presented in Table 1. The frequencies of detachments in all treatments are presented as the observed exceptions relative to the total number of females. The frequency of occurrence of detachments is more complex due to non-random disjunction and different viabilities of the sexes, to mention just two factors. Rearrangements have been obtained from every treatment and sample of germ cells. In young females fractionation of treatment results in significantly fewer rearrangements ($P<0·01$) than the concentrated treatment. Anoxia during the interval results in significantly higher rearrangement frequencies ($P<0·001$) than the uninterrupted treatment.

In old females there appears to be no fractionation effect, which is, unexpectedly, the same as the results obtained in eggs laid shortly after irradiation, but the *ANA* series does not show the expected increase in rearrangements. The results for eggs laid 11–32 days are substantially in agreement with the results obtained for rearrangements produced in eggs laid 0–4 days after irradiation[6]. For purposes of comparison, a portion of the 0–4 days results are reproduced in Table 1.

The results for immature oocytes and oogonia are consistent with the notion that, for young females, these rearrangements are formed completely very shortly after irradiation, and show that they depend on oxygen for completion, and that anoxia blocks restitution and usually alters the relationship of rejoining. Furthermore, the results indicate that the age of the female at irradiation appears to influence the restitution process in her various germinal cells.

Genetic analyses demonstrated that detachments of the attached-*X* chromosomes were indeed in many cases translocations involving the major autosomes as well as the *Y* and the smaller IVth chromosome of *Drosophila*[4,5]. Other workers, for example Neuhaus[7], considered the induced detachments to be cross-overs between the *X* and *Y*.

Induced 'crossing-over' is confined to heterochromatic regions[8]. Detachments also occur there. Induced 'cross-overs' are greater in frequency in eggs laid during 6–15 days after irradiation than days 0–4. In the case of detachments more of the changes are found in eggs oviposited in the first days, as is also the case for other induced mutational events, for example, recessive lethals, dominant lethals and chromosome loss[3].

The notable dearth of chromosome translocations found induced in early oocytes and oogonia, as well as in spermatogonia, is partly due to the sorting out, in perhaps half the cases, of complementary parts of the rearrangement into different daughter cells (including a mechanism suggested by Novitski[1] that oogonial sex cells lacking an *X* chromosome may be unable to survive and do not reach the meiotic stage); but it has long been known that this loss of cases is far from enough to explain the lack of translocations obtained from these stages. The present results show that separation of the attached-*X* does not necessarily cause a lethal aneuploidy, and would permit the survival of those cells in which the detachment is rearranged in a euploid order.

The frequencies of gross chromosome rearrangements (if we may so consider them) reported here for immature germ cells in females are the highest observed, but the genetic technique is more sensitive than those used by previous workers.

This research was supported by grants from the National Science Foundation and the Rutgers University Research Council.

Seymour Abrahamson*

Department of Biology,
Rutgers University,
Newark, New Jersey.

* Present address: Department of Zoology, University of Wisconsin, Madison, Wisconsin.

[1] Novitski, E., *Dros. Inform. Serv.*, **27**, 108 (1953).
[2] Muller, H. J., and Herskowitz, I. H., *Amer. Naturalist*, **88**, 177 (1954).
[3] King, R. C., Darrow, J. B., and Kaye, N. W., *Genetics*, **41**, 890 (1956).
[4] Abrahamson, S., Herskowitz, I. H., and Muller, H. J., *Genetics*, **41**, 410 (1956).
[5] Parker, D. R., and McCrone, J., *Genetics*, **43**, 172 (1958).
[6] Abrahamson, S., *Intern. J. Rad. Biol.* (in the press).
[7] Neuhaus, M. J., *Z. Indukt. Abstamm.-u. Vererbl.Lehre*, **71**, 265 (1936).
[8] Muller, H. J., *Genetics*, **10**, 470 (1926).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT EXPERIMENTAL OFFICER (with a degree in science or horticulture, or equivalent qualification) IN THE PHYSIOLOGY SECTION, for growth and development studies on vegetable crops—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (August 5).

SCIENTIFIC OFFICER (with a good honours degree in chemistry, or equivalent qualification, and preferably with some experience of, and interest in, food technology), for investigations into the problems involved in various aspects of biscuit manufacture—The Deputy Director of Research, Baking Industries Research Station, Chorleywood, Herts (August 5).

LECTURER or ASSISTANT LECTURER IN ZOOLOGY (ENTOMOLOGY) at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 7).

EXPERIMENTAL OFFICER (graduate in agriculture, botany or agricultural botany) IN THE CEREAL AND BRASSICA BREEDING STATION, for work on the recording and handling of breeding and genetical material—The Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth (August 10).

LECTURER (preferably with research experience in surface chemistry or chemical kinetics) IN PHARMACY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 11).

LECTURER/SENIOR LECTURER IN CLASSICAL ARCHÆOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 11).

LECTURER (graduate in science or engineering) IN THE DEPARTMENT OF BUILDING SCIENCE—The Registrar, The University, Liverpool (August 12).

SENIOR RESEARCH ASSISTANT (Ph.D. in theoretical physics or equivalent research qualifications) to Prof. N. H. March, Department of Physics, for work concerned with the study of impurities and imperfections in metals and semi-conductors using density matrix methods—The Registrar, The University, Sheffield 10 (August 12).

FELLOW (specialized in organic chemistry, with special reference to the chemistry of compounds of neuropharmacological significance) IN THE DEPARTMENT OF PHYSIOLOGY, John Curtin School of Medical Research, Australian National University—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 14).

LECTURER or ASSISTANT LECTURER (with special interest in physical chemistry) IN CHEMISTRY at the University College of the West Indies—Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 14).

LECTURER or ASSISTANT LECTURER (preferably with a knowledge of tropical soils) IN SOIL SCIENCE in the Faculty of Agriculture, University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 14).

RESEARCH ASSISTANT (with a degree in chemistry, biochemistry, agricultural chemistry or a related subject) IN THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, for a research programme supported by the Agricultural Research Council on bone metabolism and egg shell formation in the fowl—The Professor of Physiological Chemistry, The University, Reading (August 14).

LECTURER (with a higher degree in science with specialization in some branch of physical chemistry, with evidence of research ability and preferably with university teaching experience) IN PHYSICAL CHEMISTRY at the University of Queensland—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 15).

RESEARCH ASSISTANTS (2) (with at least a B.Sc. degree, or equivalent qualification, and with special interest in physical chemistry or chemical engineering), to continue a study of the equilibrium properties of imperfect gases—The Registrar (Room 22, O.R.B.), The University, Reading, Berks (August 15).

SENIOR LECTURER or LECTURER (preferably with research interests in epidemiological or immunological aspects) IN PARASITOLOGY in the Faculty of Rural Science, University of New England, Armidale, New South Wales, Australia, to teach and carry out research in parasitology with particular reference to livestock aspects—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 15).

TEXTILE TECHNOLOGIST (with a first- or second-class honours degree in textile technology, chemistry or physics, or Associate or Fellow of the Textile Institute, or equivalent qualifications) at the Tropical Products Institute, London, to supervise a group concerned with research into, and laboratory examination of, various aspects of animal and vegetable fibres, pulp, paper and board-making materials, hides, skins, etc., of tropical origin—Civil Service Commission, 17 North Audley Street, London, W.1, quoting Ref. No. S/5333/61 (August 15).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF ANATOMY—The Registrar, University College, Cathays Park, Cardiff (August 18).

ASSISTANT LECTURER IN PHYSIOLOGY—The Registrar, The University, Sheffield (August 19).

CHAIR OF PATHOLOGY in Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 20).

LECTURER or ASSISTANT LECTURER (with experience and research interests in plant physiology) IN BOTANY at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 20).

READER IN ELECTRIC TRACTION at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 21).

SENIOR LECTURER or LECTURER (with a thorough knowledge of comparative and general physiology) IN ZOOLOGY at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 21).

FELLOW (Rees Jefreys Road Fund) (with an honours degree in mathematics, geography or town planning, or equivalent qualification) AT THE ROAD RESEARCH LABORATORY, Traffic and Safety Division, Langley, Bucks, to work either on the quantitative aspects of urban transport needs or traffic conditions in new towns—The Director of Road Research, Road Research Laboratory, Harmondsworth, Middlesex (August 25).

READER IN AERONAUTICS at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 29).

LECTURER or SENIOR LECTURER IN BOTANY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 30).

KEEPER OF SCIENTIFIC BOOKS (with considerable experience of university or research library administration and a good honours degree, preferably in science), to superintend the Radcliffe Science Library—The Librarian, Bodleian Library, Oxford (August 31).

LECTURER (with an honours or higher degree in chemical engineering or equivalent qualifications, and preferably with an interest in mass transfer or nuclear chemical engineering) IN CHEMICAL ENGINEERING at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

LECTURER or ASSISTANT LECTURER (preferably with qualifications in inorganic chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Secretary, Chemistry Department, University College, Science Buildings, Merrion Street, Dublin (August 31).

LECTURER IN CROP ECOLOGY at the Waite Agricultural Research Institute, University of Adelaide, South Australia, to undertake ecological or physiological studies on the lucerne crop, and to evaluate and develop lucerne ecotypes for grazing—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 31).

LECTURER (with a degree, preferably an honours degree and some industrial and teaching experience) IN MECHANICAL ENGINEERING at the University of Queensland, to conduct lectures, laboratory and drawing office work in the fields of applied mechanics (including dynamics and stress analysis) and engineering drawing and design—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 31).

READER (with special interest in the properties of materials and able to initiate and guide research into the properties of materials such as are related to future developments in electrical engineering) IN ELECTRICAL ENGINEERING at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 31).

SCIENTIFIC OFFICER (honours graduate with a special interest in plant breeding and genetics), for work on tree fruits under experienced leadership, as one of a team of fruit breeders—The Secretary, East Malling Research Station, Maidstone, Kent (August 31).

SENIOR LECTURER or LECTURER (male, British, with an appropriate honours degree) IN PHYSICS AND ELECTRICAL ENGINEERING at the Royal Naval College, Greenwich, London, S.E.10—The Director (P), Education Department, Admiralty, London, S.W.1 (August 31).

TECHNICAL OFFICER or SENIOR TECHNICAL ASSISTANT (with considerable laboratory experience and competence with apparatus and techniques in biology, and preferably with the preparation and collection of botanical teaching materials and histological methods) at the University of Adelaide, South Australia, to supervise basic laboratory apparatus and preparation of undergraduate classes—The Registrar, The University of Adelaide, Adelaide, South Australia (August 31).

ASSISTANT LECTURER IN MATHEMATICS (PURE OR APPLIED)—The Principal, Royal Holloway College, Englefield Green, Surrey (September 1).

LECTURER IN THE DEPARTMENT OF MEDICAL PHYSICS—The Secretary, Royal Free Hospital School of Medicine, 8 Hunter Street, London, W.C.1 (September 1).

SENIOR LECTURER IN APPLIED ELECTRONICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 1).

LECTURER or ASSISTANT LECTURER (medically or non-medically qualified candidate) IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/N (September 4).

LECTURER or JUNIOR LECTURER IN PURE MATHEMATICS—The Registrar, Trinity College, Dublin (September 4).

LECTURER (with experience of modern physical methods of structure determination, and especially of those involving electric birefringence, dipole moments, etc.) IN PHYSICAL-ORGANIC CHEMISTRY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 8).

LECTURER IN SOCIAL ANTHROPOLOGY at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, September 8).

ASSISTANT DIRECTOR OF THE BRITISH INSTITUTE OF HISTORY AND ARCHÆOLOGY, Dar es Salaam, Tanganyika, to combine research with the supervision of the Institute's library and to share in the direction of students—Secretary, British Institute of History and Archæology in East Africa, c/o British Academy, Burlington Gardens, London, W.1 (September 15).

CHAIR OF PHILOSOPHY in the University of Ceylon—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Ceylon and London, September 15).

LECTURER OR ASSISTANT LECTURER IN PARASITOLOGY (MEDICAL ENTOMOLOGY) at the University of Malaya, Singapore—The Secre-

tary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 15).

READER or SENIOR LECTURER IN AGRICULTURAL CHEMISTRY; LECTURER IN AGRICULTURAL CHEMISTRY; and a LECTURER IN ANIMAL HUSBANDRY in the Faculty of Agriculture, Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 15).

SENIOR LECTURER or LECTURER (well qualified in electrical engineering or physics, with general experience in electronics and a considerable detailed knowledge of solid-state devices and circuits) IN ELECTRICAL ENGINEERING at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (September 15).

CHAIR OF STATISTICS tenable in the Newcastle Division of the University—The Registrar, Durham University Office, 1A Kensington Terrace, Newcastle-upon-Tyne 2 (September 25).

HISTOCHEMIST, Scientific Officer grade (with a first- or good second-class honours degree and preferably with some research experience in a related field), for research into mechanisms of insecticidal action using histochemical methods—The Director, Pest Infestation Laboratory, London Road, Slough, Bucks (September 30).

SCIENTIFIC OFFICER (honours graduate), for research on problems arising out of the vegetative propagation of fruit plants—The Secretary, East Malling Research Station, Maidstone, Kent (September 30).

SENIOR LECTURER IN PHYSICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

ASSISTANT, Grade B (qualified to teach to G.C.E. advanced level), IN BIOLOGY—The Principal, Percival Whitley College of Further Education, Francis Street, Halifax.

ASSISTANT, Grade B, IN THE DEPARTMENT OF PHYSICS—The Principal, College of Technology, Huddersfield.

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER (with previous experience in virological techniques, preferably including tissue culture), for work connected with production of attenuated strains of foot-and-mouth disease vaccines—The Secretary, Research Institute (Animal Virus Diseases), Pirbright, Surrey.

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree in physics or physical chemistry or H.N.C., or equivalent qualification), to work on physical problems on the processing of foodstuffs—The Director, Low Temperature Research Station, Downing Street, Cambridge.

BIOCHEMIST or BIOCHEMICAL ASSISTANT, to assist with metabolic research—The Director, Medical Unit, King's College Hospital Medical School, Denmark Hill, London, S.E.5.

CHEMIST (with research experience in physical or bio-chemistry), to work on certain aspects of the chemistry of wheat protein—The Director of Research, Cereals Research Station, Old London Road, St. Albans, Herts.

LECTURER (preferably with a higher degree and industrial research experience) IN METALLURGY in the Department of Materials, to lecture at postgraduate level in his own special subjects; to supervise student research work, and to assist in initiating and directing long-term programmes of research, some of which may be externally sponsored—The Recorder, College of Aeronautics, Cranfield, Bletchley, Bucks.

METALLURGIST, Scientific Officer grade (with an honours degree), at the Royal Mint, to assist in supervision of production, investigation of problems of materials and manufacture, assaying and general research in the fields of physical metallurgy and metallography—Establishment Officer, Royal Mint, London, E.C.3.

POSTDOCTORAL RESEARCH FELLOW IN THE THEORETICAL PHYSICS INSTITUTE—L. E. H. Trainor, Physics Department, University of Alberta, Edmonton, Alberta, Canada.

RESEARCH ASSISTANT (preferably with experience in cytology), to take part in a programme designed to study human chromosome abnormalities and associated disorders—The Secretary, Genetics Department, The University, Sheffield 10.

RESEARCH ASSISTANT (graduate in physics, with research or industrial experience) IN LOW-TEMPERATURE PHYSICS, to work under the direction of Dr. Yates, on the determination of the thermal constants of materials at liquid-hydrogen temperatures—The Registrar, Bradford Institute of Technology, Bradford 7.

RESEARCH ASSISTANT (recent graduate in physics) IN THE DEPARTMENT OF PHYSICS, for work on track analysis in connexion with the National Bubble Chambers project—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15.

SENIOR LECTURER (with a good honours degree and appropriate teaching and/or industrial experience) IN HIGH POLYMER CHEMISTRY—The Principal, Rutherford College of Technology, Northumberland Road, Newcastle-upon-Tyne 1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Scientific and Industrial Research. Report of the Research Council for the year 1960. Pp. 65. (Cmnd. 1365.) (London: H.M. Stationery Office, 1961.) 4*s.* net. [295

The International Council for Bird Preservation. British Section. Annual Report for 1960. Pp. 38+4 plates. (London: International Committee for Bird Preservation, British Section, c/o British Museum (Natural History), 1961.) 3*s.* [305

Department of Scientific and Industrial Research. Report of the National Chemical Laboratory 1960: Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for 1960. Pp. iv+70+4 plates. (London: H.M. Stationery Office, 1961.) 4*s.* 6*d.* net. [305

British Drug Houses, Ltd. Moisture Determination by the Karl Fischer Reagent. Second edition. Pp. 15. (Poole, Dorset: British Drug Houses, Ltd., 1961.) [305

University of Glasgow Appointments Committee. Report for the year ended 31st December, 1960. Pp. 19. (Glasgow: The University, 1961.) [305

Ministry of Education: Science Museum. Books on the Chemical and Allied Industries: a subject Catalogue of Books in the Science Library—Short-title List of Books published 1930 and onwards on the Chemical Industry, with Books on Mining and Metallurgy, and the Paper, Leather and Textile Industries. Pp. ix+118. (London: H.M. Stationery Office, 1961.) 12*s.* 6*d.* net. [305

Science Museum, London. Science Library Bibliographical Series No. 781: Books on the Chemical and Allied Industries. Pp. 10. (London: Science Museum, 1961.) [305

Other Countries

International Atomic Energy Agency, Vienna. Services and Technical Assistance Available from the I.A.E.A. Pp. 42. (Vienna: International Atomic Energy Agency, 1961.) [245

Food and Agriculture Organization of the United Nations. F.A.O. Picture Sheet Story: Why a Freedom from Hunger Campaign? Pp. 2. (Rome: Food and Agriculture Organizations of the United Nations, 1961.) [245

Mauritius Sugar Industry Research Institute. Annual Report, 1960. Pp. 106+xxxviii. (Reduit: Mauritius Sugar Industry Research Institute, 1961.) [245

Annals of the New York Academy of Sciences. Vol. 93, Article 1. Vertical Take-off and Landing (VTOL) Rotorless Aircraft with Inherent Stability. By L. B. Laskowitz. Pp. 1–24. (New York: New York Academy of Sciences, 1961.) [245

The Year Book of the International Council of Scientific Unions, 1961. Pp. 110. (The Hague: International Council of Scientific Unions, Paleis Noordeinde, 1961.) [245

Skrifter fra Danmarks Fiskeri-og Havundersøgelser, Nr. 21. Fiskriumdersøgelser I 1960 ved Danmark, Faeroerne og Grønland. Ved E. Bertelsen og Paul M. Hansen. Pp. 114. (København: J. Jørgensen & Co., 1961.) [245

Museum of Applied Science of Victoria. Report of Activities for the year ended 30th June, 1960. Pp. 23. (Melbourne: Museum of Applied Science of Victoria, 1961.) [245

Western Australia: Government Chemical Laboratories. Report for the year 1959. Pp. 27. (Perth: Government Printer, 1960.) [245

Hémostase: Revue Internationale de l'Hémostase, de la Coagulation, des Maladies Hémorragiques et des Thromboses. Vol. 1, No. 1. Pp. 102+xlvii. Revue trimestrielle. Subscription (12 months): 65 N.F.; Students' Tariff 40 N.F. Single Issues: 20 N.F.; Students' Tariff 14 N.F. (Paris: S.P.E.I., 14 Rue Drouot, 1961.) [245

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 60–62: Belcher Islands, Northwest Territories, 33M, 34D,E. By G. D. Jackson. Pp. ii+13+map. (Ottawa: Queen's Printer, 1960.) 50 cents. [305

National Academy of Sciences—National Research Council. Nuclear Science Series. Report No. 34: Radioactive Contamination of Materials used in Scientific Research. By James R. DeVoe. Pp. vii+142. (Publication 895.) (Washington, D.C.: National Academy of Sciences—National Research Council, 1961.) [305

United States Department of the Interior: Geological Survey. Bulletin 1089–A: Geology of the Alvord Mountain Quadrangle, San Bernardino County, California. By F. M. Byers, Jr. Pp. iv+71+6 plates. Bulletin 1111–E: Stratigraphy of the Moose River Synclinorium, Maine. By Arthur J. Boucot. Pp. iii+153–188+plate 34. (Washington, D.C.: Government Printing Office, 1960 and 1961.) [305

Durban Museum Novitates. Vol. 6, Part 6 (20th March 1961): Miscellaneous Taxonomic Notes on African Birds, XVI. By P. A. Clancey. Pp. 79–104. 5*s.* net. Vol. 6, Part 7 (15th April, 1961): Notes on South African Forms of the Collared Sunbird *Anthreptes collaris* (Vieillot), with the description of a New Race. By Michael P. Stuart Irwin. Pp. 105–111. 20 cents. (Durban: Durban Museum, 1961.) [305

Deutschen Wetterdienstes. Mitteilungen des Deutschen Wetterdienstes. Nr. 24 (Band 4): Die Kraftwirkung Elektrisch Geladener Eiskristalle auf Aerosolteilchen. Von Paul Hess und Johannes Tauscher. Pp. 24. Nr. 25 (Band 4): Über Zusammenhänge Zwischen Parametern des Luftdruck- und Temperatur-Feldes und dem Niederschlag. Von Hans Mollwo. Pp. 36. Berichte des Deutschen Wetterdienstes. Nr. 73 (Band 10): Beiträge zum Internationalen Geophysikalischen Jahr, 1957/58. 1: Mess-Ergebnisse und Berechnungen zum Wärmehaushalt der Erdoberfläche. Von Ernst Frankenberger. Pp. 41+10 tabellen im Anhang. 10.50 D.M. Nr. 74 (Band 10): Der Einfluss der Bodenbedeckung auf das Klima des Bodens und der Bodennahen Luft. Von Franz Fuss. Pp. 38. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1960 und 1961.) [305

Académie Royale de Belgique. Classe des Sciences. Mémoires. Tome 32, Fascicule 5: Le Problème Naturel aux Valeurs Propres. Par Jean Kestens. Pp. xiv+122. (Bruxelles: Académie Royale de Belgique, 1961.) 120 francs. [305

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office